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Interactions Between Soil Minerals and Microorganisms

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I. INTRODUCTION

Soils are complex living media and contain large amounts of microorganisms (e.g., 10^7 bacteria, 10^6 actinomycetes, 10^5 fungi, 10^5 protozoans, and 10^4 algae per gram of oven dried soil). Where are these microorganisms located? What are their relations with soil minerals? What are the main limiting factors in relation to these constituents that govern the development and growth of the microorganisms? These are questions that are very difficult to answer, both for microbiologists and for soil scientists. However, answers to these questions are needed, both for fundamental knowledge in microbial ecology and for applications in agronomy, forestry, and plant pathology. These questions also need to be answered if microorganisms are to be introduced into soils.

In this chapter, we aim to introduce some elements of discussion for soil scientists and to indicate new trends in research for the future. This review is not exhaustive, and we focus on only some of the interactions between soil minerals and microorganisms.

Interaction, as used in this chapter, means an action of the physical and chemical environment on the growth and development of microorganisms (e.g., supplying oxygen, water, nutrients, or toxins; Figure 1). It also means an action of the microorganisms on soil constituents, namely, weathering, release of elements, aggregation, and transformation of soil organic matter.

In the first section, new information is presented about soil constituents, their organization, and their reactivity, which is necessary

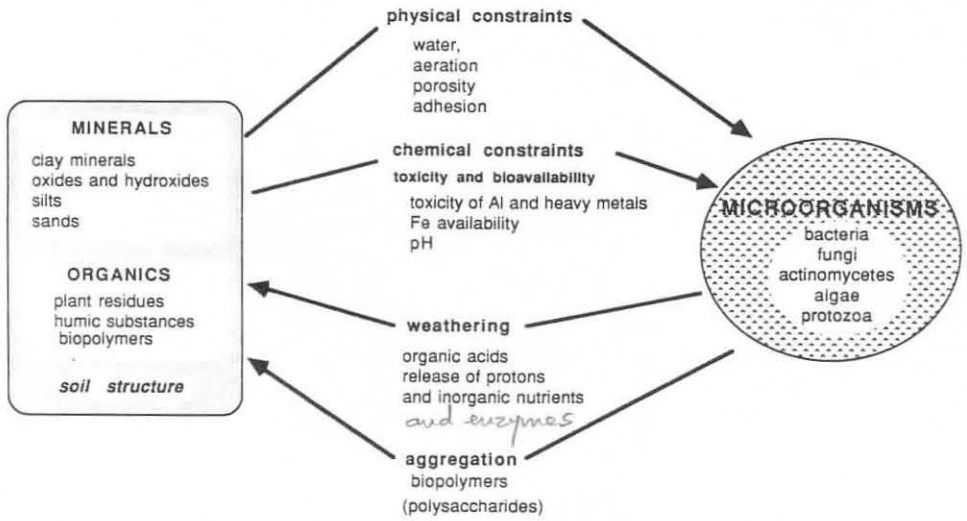


Figure 1 Some interactions between soil constituents and microorganisms.

to better understand both the results of experimental studies and the phenomena that occur in the field. In the second section, selected interactions are presented. Two important environmental factors have been chosen to represent stresses for microbial life: a physical one (i.e., the role of water potential); and a chemical one (i.e., metal availability and its toxicity). The action of microorganisms on soil constituents through their role in aggregation and "weathering" processes will also be considered. Interactions between microorganisms and soil organic matter are not considered, as reviews exist on this subject [e.g., 1].

II. SOIL MINERAL CONSTITUENTS

A distinction is made between (1) the soil constituents considered as individual components (soil texture), which, for the finer and more active ones, corresponds to what is called the *colloidal level*; and (2) the natural association of these constituents (soil structure), which results in porosity, the *aggregate level*.

Most studies on interactions between minerals and microbes, whether under laboratory or field conditions, have been conducted at the colloidal level. The aggregate level, which appears to be predominant in several interactions, such as the resistance of

microbes to drying, has to be viewed with special attention and as being considerably more complex than the colloidal level. Aggregate organization is more complicated than the model of Hattori [2], which was the first one proposed.

A. Constituents as Individuals: The Colloidal Level

Fractionation of Soil Constituents

The best way to determine the relative importance of soil constituents is to separate them according to their size. Therefore, mechanical analyses have been extensively developed for the study of the mineral constituents of soil, and these methods have been recently applied to the other constituents.

The classic granulometric methods, the purpose of which is to determine the true size of individual mineral particles, use chemical reagents to remove organic matter (OM) and amorphous compounds (H_2O_2 and reductive agents) and to disperse the clay minerals (K^+, Na^+, NH_4^+). If chemical reagents are avoided, the particle size separation of both mineral and organic constituents can be achieved by the addition of mechanical energy only.

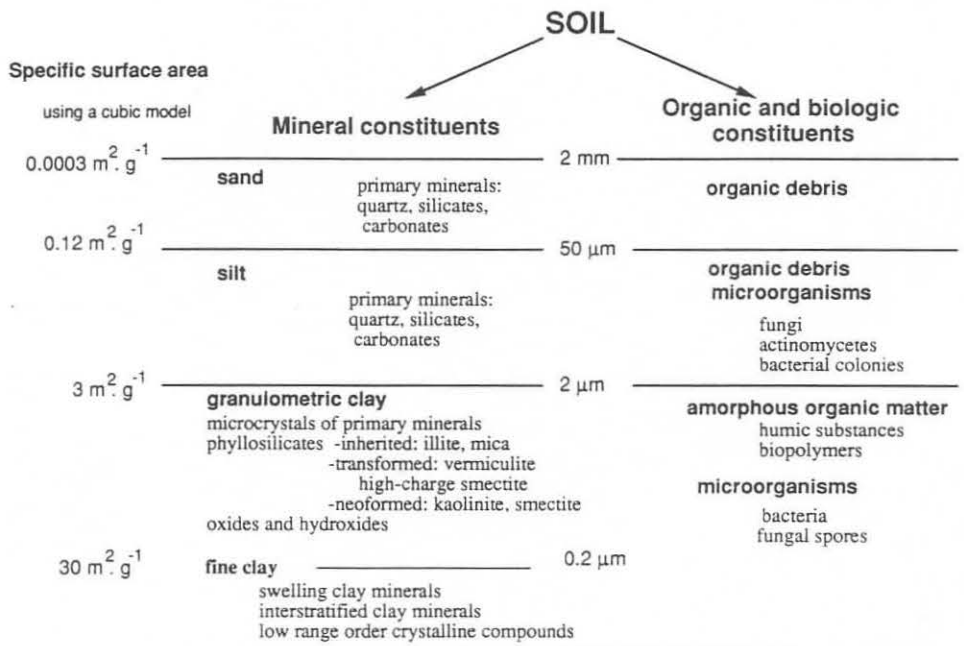
For mineral constituents, separation can be achieved between the >2-μm fraction, which is composed of sand and silt, and the <2-μm fraction, which is the "granulometric" clay (Table 1). The former fraction represents the "inert" skeleton of soil, and the latter fraction represents the active fraction, especially if sorptive interactions are considered.

An important part of the <2-μm fraction is composed of micro-divided primary minerals (e.g., calcite, quartz, feldspars), which do not have the structure and properties of clay minerals. The "mineralogical" clay consists of phyllosilicate minerals, with specific structures and small particle sizes that confer specific chemical and physical properties. Oxides or hydroxides of Al, Fe, Ti, and Mn, with either a long- or a short-range crystalline structure, may also modify the clay properties.

Size fractionation demonstrates the very small size of clays that occur naturally in soil. The finer the fraction, the greater the surface area and swelling properties and the less the crystallinity [3].

The fractionation can be carried further on the basis of size, density, or magnetic properties. Size fractionation, combined with density, permits more accurate separation of oxides, clays, and OM. High field magnetic separation [4] enables the separation of clays on the basis of their chemical composition. Hence, classic fractionation methods, combined with new methods, such as high-resolution transmission electron microscopy (HRTEM), offer a new trend in research.

Table 1 Size Fractionation of Soil Constituents



Organic and biological constituents can also be fractionated according to size after dispersion by mild agitation [5], ultrasonic treatment [6,7], or ion exchange on Na-resins [5,8,9]. Ramsay [10] used agitation or ultrasonication for the enumeration of bacteria by direct count and autoradiography. It must be emphasized that none of the size fractionation methods based on mechanical disruption results in the complete dispersion of the constituents. This indicates the presence of stable aggregates and the presence of organisms located inside or outside the aggregates [2].

Organization and Physicochemical Properties of Soil Constituents

Structure, Size, and Organization of Minerals. The sand and silt fractions are composed mainly of primary minerals, such as quartz and feldspars. When phyllosilicates are present, they are monocrystals (micas and chlorites) with low surface area; only vermiculites and zeolites, which are very rare, can have specific chemical

properties (e.g., high cation-exchange capacity; CEC). Gypsum and calcite are an important source of Ca, as the result of their solubility (3 g L^{-1} for $CaSO_4 \cdot 2H_2O$ and 20–100 mg L^{-1} for calcite). Depending on the size and shape of primary minerals, their arrangement can result in different overall porosities and range of pore sizes.

Since the beginning of the 20th century, X-ray diffraction has been the main method for the identification of clay phyllosilicates through the evaluation of the elementary layer size and the apparent displacement of the layer spacing by water or polyalcohols [11] (Table 2). This kind of identification, based on the layer and interlayer space, can help predict chemical properties, but it is important to know more about the organization of the clay, particularly at the particle level. The organization of clay phyllosilicates has been the subject of major studies in the last 10 years [12,13], based on the characterization of wet samples or samples the fabric of which is preserved in its natural state of humidity [14]. The development of new methodologies (e.g., low-angle X-ray scattering and electron microscopy) has made it possible to evaluate the number of layers per particle, their lateral extension, their mode of association [11], and to provide information on what is called the "texture" of the clay [15].

On the basis of such criteria, it is possible to better understand the behavior of the smectites in comparison with those of all the other clays. Smectites have an organization with variable geometry, the variation being dependent on the crystallochemical composition, the type of charge-compensation cations, the water content, the osmotic pressure (see under Section III.A), and the history of the clay. Only alkaline cations (e.g., Na, K, Li) at low concentration result in the development of a diffuse layer between the clay plates and, thus, in gel formation (layers separated by 2–15 nm) or in complete dispersion of tactoids composed of one to five layers, which correspond to the fundamental particles [16]. Other cations (e.g., Ca, Mg) give a quasicrystal organization [17], in which the interlayer space goes from an upper limit of 1 nm (four layers of water), which is the normal state under soil conditions, to 0.4 nm, which corresponds to air-drying (about 40% relative humidity; RH), or to even complete collapse under vacuum. Layers are associated in a subparallel manner (face-to-face), and their number ranges from 50 to more than 200, with decreasing water potential (decreasing water content) [18]. Such particles are highly deformable, and they delimit a three-dimensional porosity, the size of which depends on the size of the particles (0.5–2 μm) (Figures 2 and 3). With Ca or Mg cations, each drying leads to an irreversible increase in the number of layers.

Table 2 Classification of Clay Minerals and Major Surface Properties

Type of clay mineral	Layer type (nm)	Layer spacing Ca[a] (nm)	Amount of charge (negative)[b]	Cation-exchange capacity (mEq Kg^{-1})	Surface area (m^2/g)	Dominant type of charge
Kaolinite	0.7	0.7	No charge or very low	10–200 silanol groups	5–200 External	Variable
Halloysite		1				
Illite (I)	1	1	0.6–0.9 (K)	100–400	50–100 Ext. + int.	Permanent (–)
Interstratified (I:S)	1	1–1.7	0.9–0.3 K-Ca	100–800	100–800 Internal	Permanent (–)
Smectite (S)	1	1–1.7	0.25–0.6 Ca	800–1300	800 Internal	Permanent (–)
Vermiculite	1	1–1.4	0.6–0.9 Ca	1300–1800	800 Internal	Permanent (–)
Hydroxy-Al-vermiculite	1	10–1.4	0.6–0.9 [Al(OH)n]	100–400 100–400	Partially internal	Variable and permanent
Chlorite	1 + 0.4	1.4	No charge	10–200	5-20 External	Variable

[a]With water on polyalcohol.
[b]For Si_4O_{10} in equivalent.

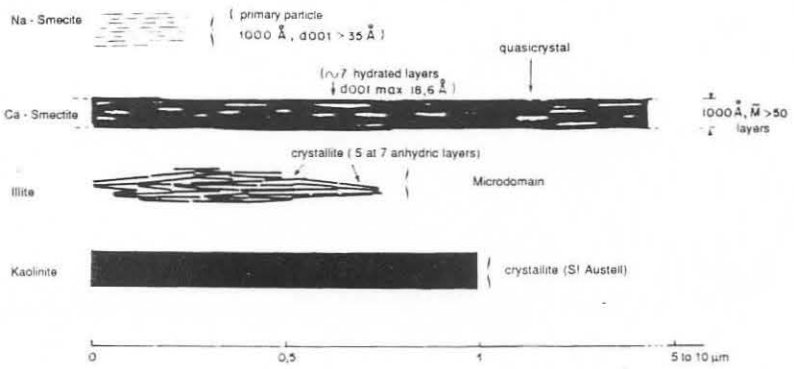
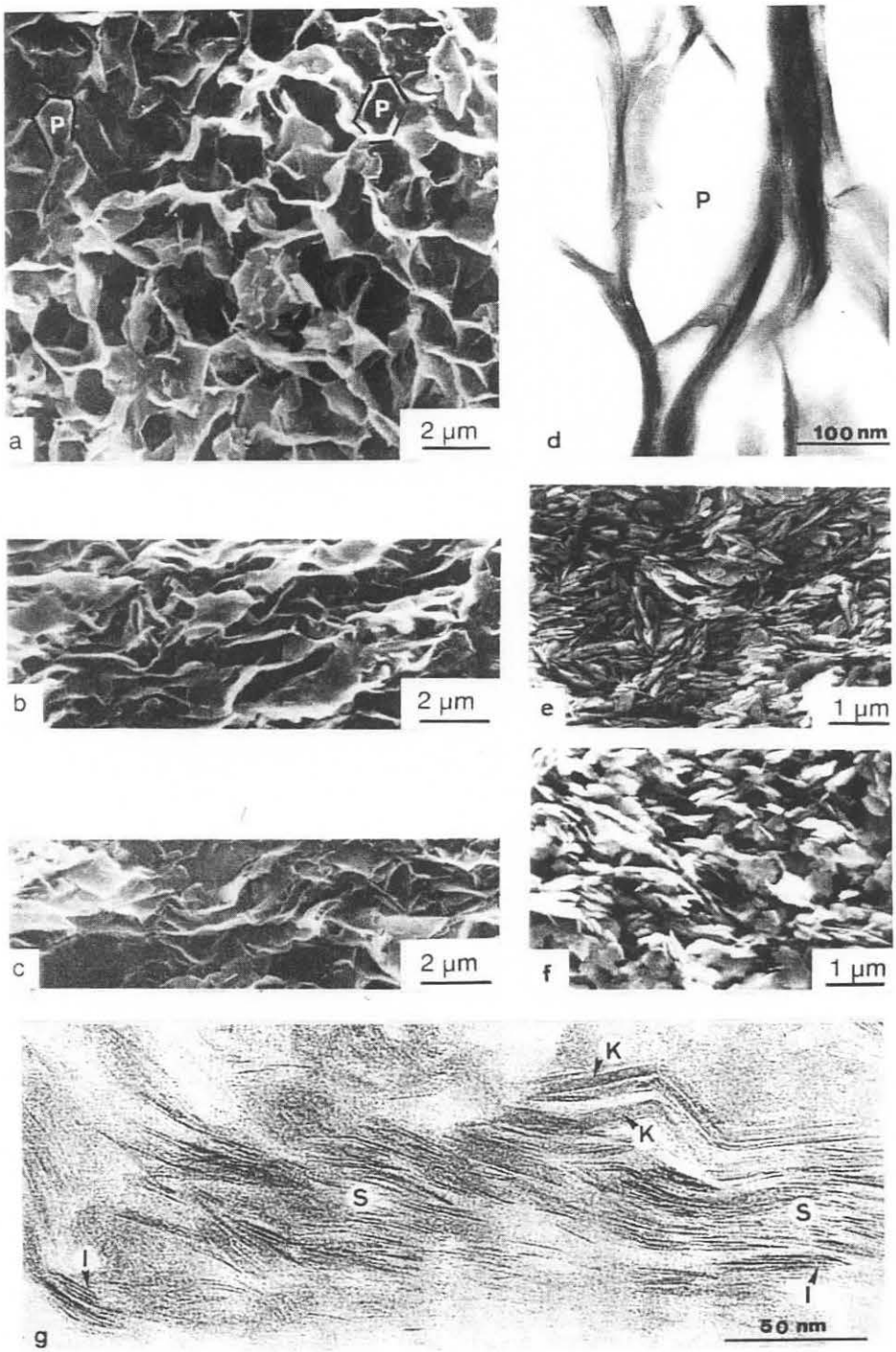


Figure 2 Organization of clay particles. From Ref. 12.

Other clays present a more stable organization in which a definite number of layers are built into rigid particles or crystallites (see Figures 2 and 3). If reference minerals derived from quarries are considered, illites and kaolinites have more than ten layers, and their lateral extension is important (Figure 2). Recent studies [3] have shown that in clays of soils and sediments, very small particles (one to ten layers) with limited lateral extension can occur (see Figure 3). Such clay can often be considered as *Texturally interstratified*, that is, superposition of very small particles with different layers that may be collapsed or opened. Minerals with 1-nm spacing (illites) are very abundant in temperate regions and correspond to two types of clay crystals: thick crystals of micromicas with several packs of layers; or very small particles with fewer than five layers, which are generally associated face-to-face to form domains. These are quite similar to small aggregates, but they correspond to more oriented clays.

Soil smectites that have mainly a beidellitic composition [19,20] are also quite different from reference minerals used in the laboratory, as these soil particles have a very small lateral extension and have always some collapsed layers [3]. A main characteristic of soil clays is a relatively high ratio of external (interparticle) to internal (interlayer) surface area. This ratio has to be considered in interactions of clays with polymers and microorganisms.

The difference observed in particle organization between smectites and other clays may contribute to the explanation of the specific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or even unique role of montmorillonite [1]. The properties of other clays, which are rigid, are more like those of silt particles, but the finer the size, the greater will be the surface properties and possible reactions with microbes.

Surface and Exchange Properties of Soil Minerals. The physicochemical properties of clay are governed by two main characteristics: surface area and electrical charge. Both are of major importance to biological activity.

Two kinds of surface area with different reactivities have to be distinguished: external surface, which corresponds to the surface of crystallites or particles; and internal surface, which corresponds to interlayer spaces.

External surface area decreases with an increase in particle size (see Table 1). Particles smaller than 2 μm have surface areas that exceed a few square meters per gram. External surface is the main surface for kaolinites, illites, and chlorites. Small particles of soil clays can have values up to 100 $m^2\ g^{-1}$. For smectite, the determination of external surface area is very difficult and has no relevance if the clay is dried before the determination (which is the normal way when N_2 is used).

The internal surface area increases in interstratified illite–smectite, and becomes very large in smectites and vermiculites (700–800 $m^2\ g^{-1}$) (see Table 2). Calcium-saturated smectites have an interlayer space limited to 0.8 nm, which can accommodate small polymers (e.g., polymerized Al, proteins, polyalcohols, polysaccharides). If the diffuse double layer is active (e.g., saturation with monovalent cation), the size of the interlayer has no limit and can even accommodate enzymes (>5 nm). However, such conditions will be scarce in soils, and interparticle sites are certainly the most common for polymers.

Figure 3 Clay microorganizations characterized by low-temperature scanning electron microscopy (a–c,e,f) and high-resolution transmission electron microscopy (d,g), at different water potentials. (a) Ca-Wyoming montmorillonite, −0.0032 MPa; (b) Ca-Wyoming montmorillonite, −0.1 MPa; (c) Ca-Wyoming montmorillonite, −1 MPa; (d) Ca-Wyoming montmorillonite, −0.0032 MPa; (e) St. Austell kaolinite, −0.1 MPa; (f) St. Austell kaolinite, −0.0032 MPa; (g) textural interstratified soil clay mineral. (P, pores; S, smectite layer; K, kaolinite layer; I, illite layer). (Photographs courtesy J. Berrier and A. M. Jaunet.) (a)–(f) from Ref. 12, (g) M. Robert, unpublished.

Two different types of electrical charge also need to be distinguished: a permanent negative charge and a variable charge. The first type is present mainly in 2:1 phyllosilicates, although it can exist in 1:1 minerals. It originates from isomorphic substitutions by trivalent cations (e.g., Al^{3+}, Fe^{3+}) in the tetrahedral sheet or by divalent cations (e.g., Mg^{2+}, Fe^{2+}) in the octahedra. Values of negative charge range from one charge in mica for each Si_4O_{10} unit cell (where the charge is blocked by nonexchangeable K) to 0.3 charge in smectites (see Table 2). If some negative permanent charge occurs in samples of kaolinite and halloysite, the presence of some 2:1 phyllosilicate layers has to be suspected, especially when CEC values are high [20].

Organic matter also has important exchange properties. Once the COOH or OH groups are dissociated, the charge can be considered as permanently negative, especially if the pH remains above the pKa. This charge ranges in extent similar to that for mineral constituents (1 to more than 2 mEq g^{-1}). The respective role of clay and organic matter in soil chemical properties will depend on their relative proportions.

The variable charge of minerals has received increasing attention in recent years. It originates from broken edges on the crystals and the dissociation Si-OH (silanols) or $Fe(OH)_3$ and $Al(OH)_3$. Such dissociation of $Al(OH)_3$ or $Fe(OH)_3$ depends on the pH, with the general reaction, $M(OH)_2^+ \longleftrightarrow M(OH) \longrightarrow MO^- + H_2O$, where M is the metal. Variable charges are found at the surfaces of oxides, hydroxides, and the edges of phyllosilicates, especially kaolinites. The smaller the particle size, the higher the charge. This explains the important role of short-range crystalline compounds that consist of particles of 5–10 nm for allophanes or even less for ferrihydrite or Al polymers. Fulvic and humic colloids have similar sizes. All these compounds can exist independently, but most often they are associated as coatings on clay surfaces (Figure 4) [22–24] or as clay–OM complexes, which appear as more diffuse (see Figure 4). At a pH lower than the zero point of charge value (ZPC), the overall charge is positive, but it changes to negative above this value. Hence, soils with variable charge will most often have a positive charge. This normal behavior can be modified by the specific adsorption of anions, such as SiO_4, PO_4^{3-}, or organic matter onto these positive charges. Coordination bonds can develop and can decrease the overall ZPC value of the complex and give a high-energy bond to the adsorbed compounds (e.g., PO_4^{3-}). Fixation of Al or Fe polymers on 2:1 phyllosilicates surfaces can result in a complicated situation, with a variable charge on the external surface and a permanent negative charge on the internal surface.

Soils with a permanent negative charge are generally formed in temperate regions (except for vertisols), whereas soils with a

variable charge are generally formed in tropical areas and on volcanic rocks (except for podzols). In the case of soils with variable charge, upper horizons rich in OM can have a net negative charge, and B horizons, a net positive charge at the same soil pH [25].

Although the main mineral constituents determine general soil properties, the minor compounds are very important. For example, the presence of OM and 2:1 layer clays in kaolinitic soils is very important for cation fixation and, especially, for K fixation [26], and fixation of polyvalent cations on 2:1 minerals can change many physical and chemical properties [27,28].

Usually, there is a good correlation between the CEC and total surface as determined by ethylene glycol monoethyl ether (EGME), and such characteristics are good indicators of soil behavior [29]. These surface and exchange properties of clays are very important in relation to biological processes. Until recently, the importance of interlayer spaces was emphasized. However, essentially only cations and small polymers can be fixed on interlayer surfaces [30], and larger polymers (e.g., humic compounds), most enzymes, and microorganisms adsorb only on the external surfaces [31]. This is especially true for soil clay particles that are very short and thin. In the majority of soils developed in temperate regions, microorganisms, which are negatively charged colloids [32], will be in contact with negative clay surface, unless Al or Fe polymers are present at the interface. In soils with variable charge, exposed surfaces will be mostly positively charged.

In chemical properties (e.g., CEC, surface area), smectite and vermiculite have similar exceptional properties, but these clays differ mainly in physical properties. Such differences could be useful in devising experiments aimed at determining the relative importance of chemical and physical factors in biological processes.

Physical Properties of Soil Minerals. Definitions and Methods. The status of water in soil is defined by the concept of water potential. This potential indicates the energy by which water is retained by soils or clays. Hence, it is relevant for organisms, as they have to provide equivalent energy to utilize and extract the water. It covers two terms: the *matric* and the *osmotic* potential. The former is predominant in most soils, as the solute concentration of the soil solution is generally $<10^{-2}$ M. Water retention is related to water potential and is increasingly expressed in the percentage volume, rather than the percentage weight [12]. The *water ratio* is the ratio of the volume of water to the volume of solid, and the *void ratio* (e) is the ratio of the volume of voids to the volume of solid [33].

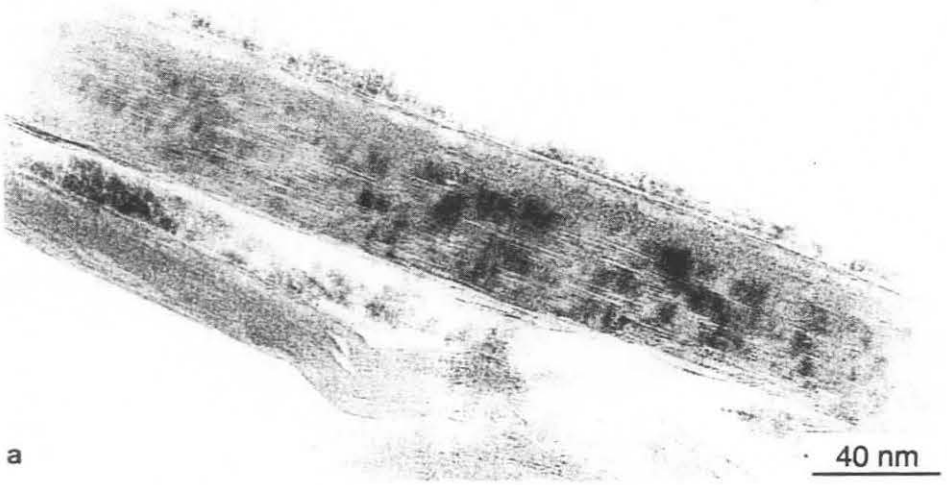
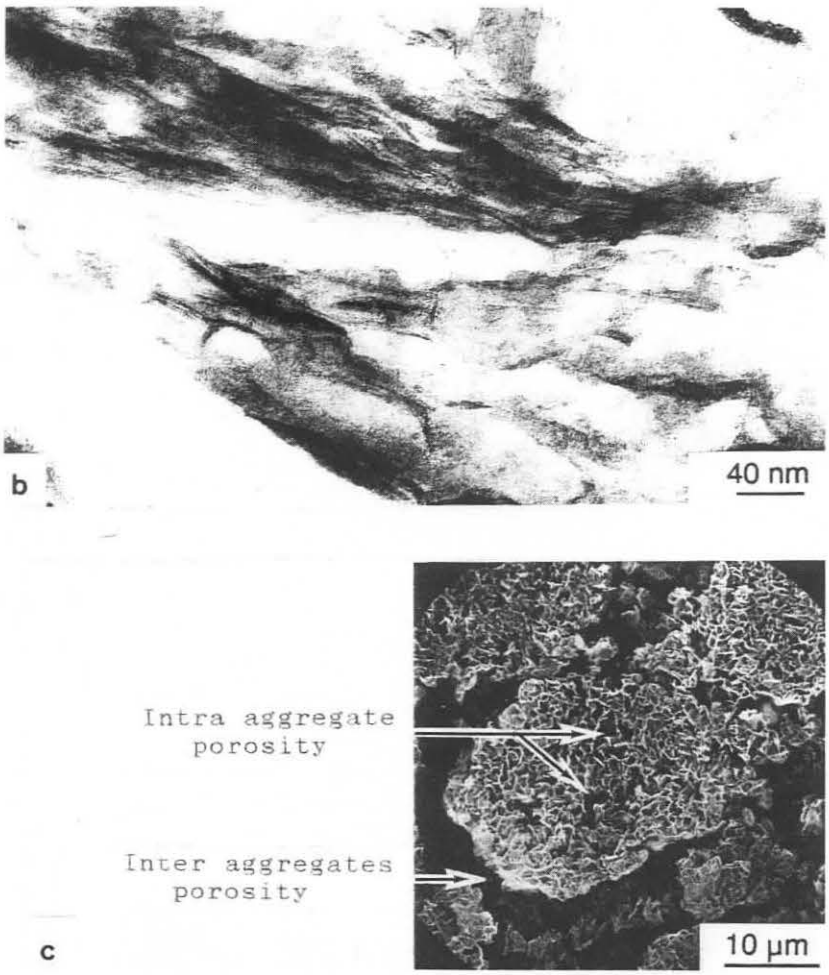


Figure 4 Clay coatings observed by high-resolution transmission electron microscopy. (a) Fe coating on Wyoming montmorillonite; (b) organic coating on smectite in a Vertisol (thin section fixed with OsO_4, and stained with lead citrate and uranyl acetate); (c) aggregates of Fe polycations-montmorillonite associations. (Photographs courtesy J. Berrier and A. M. Jaunet.) (a) and (c) from Ref. 24; (b) M. Robert, unpublished.

The monitoring of water potential is very important to better understand clay–microorganism interactions. However, true control of water potential is difficult to maintain and can very often alter biological functions. The method most generally used in laboratory experiments to establish a matric water potential is to add specific quantities of water to soil samples, this is correct unless the moisture release curve has been previously determined [34,35]. When desiccators and saline solutions are used (i.e., isopiestic control) [e.g., 36], it is difficult to renew the nutrients.

The use of polyethylene glycol is possible over only a short range of water potential, and it generates problems of aeration [37]. Suction or pressure plates are widely used [e.g., 38,39] and cover a −2 to −0.032 MPa water potential range. However, sterility of the plates is not easy to attain, although it is possible.

For high water potentials (which are probably the most important in biology) Tessier and Berrier [40] have developed a very



convenient technique (Figure 5). It consists of a filtration device that can be made of glass or polyvinyl chloride (PVC). The clod of soil or sample of clay is placed on a 0.2-µm filter, which assures the maintenance of sterility. The sample is in equilibrium with a solution that can be water, a nutritive solution, or a solution adjusted to a given osmotic pressure. The matric potential is applied by pneumatic air pressure in the range of −0.01 to −0.1 MPa. In such a system, the problem of aeration does not arise.

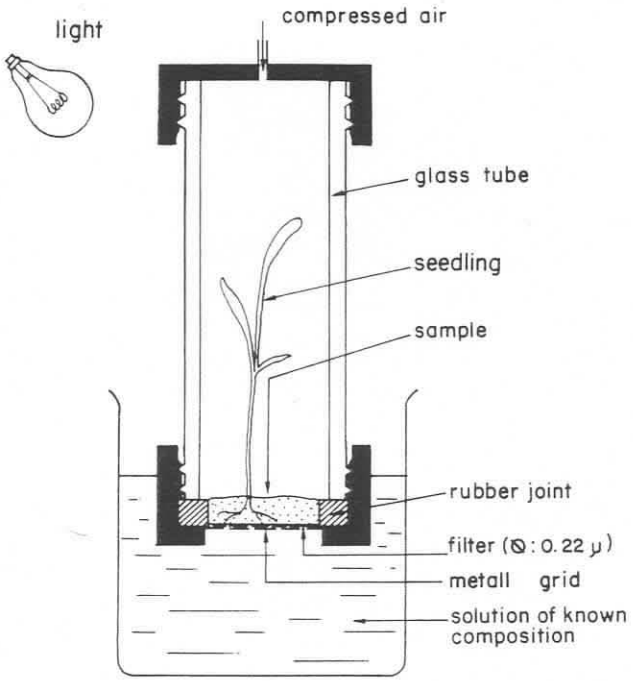


Figure 5 Filtration device for the control of high water potentials. The sample can be a clay paste or a soil clod, it can be inoculated with microorganisms, or plants can be grown. Adapted from Ref. 40.

By measuring both the weight of the water at 110°C and the volume (e.g., with kerosene [41]) of the clay or soil sample, water and air indices can be deduced [13]. The changes in these indices' evolution upon dehydration and rehydration give information on the different types of porosity and their filling by air or water. These are certainly among the best parameters that can be measured on unperturbed samples (clods of a few cubic centimeters) to characterize the physical environment of microorganisms. Other methods, such as Hg or N porosimetry, give good values for the different types of pores (both quantity and diameters from 100 mm to 10 nm), but the measurements are made on dry samples with high vacuum, thereby causing strong modifications in many soil types.

Water Retention and Porosity of Clays. In most soils, clays have the predominant role in the water retention of soils. The organization

of the clay itself depends on the water content. Thus, the entire clay–water system needs to be characterized [13]. All clays with rigid particles or crystallites (e.g., kaolinite–illites) have a simple behavior (Figure 6). At high water potential (−0.1 MPa), they retain relatively large amounts of water in a porosity that is less than 1 μm between particles. This water is readily available to microorganisms. With further increases in water potential, particles come in contact, and the porosity is then filled with air. The quantity of water retained, the energy of water retention, and the point of air entry depend on the size of the particles or domains. The water retention properties of smectites are different in intensity from those of other clay minerals, which, relative to microbial activity are mainly the following (Figures 6 and 7):

1. The amount of water retained is very high at low suctions (i.e., at low pF and high water potential): as much as seven times their weight in water for Ca-montmorillonite and 15 times for Na-montmorillonite.
2. Most of the water, especially for Ca-montmorillonite, occurs in pores between the quasicrystals or tactoids, rather than in the interlayer spaces. With −1.6 MPa (pF 4.2) as a reference, the interlayer water of Ca-montmorillonite is not available to microorganisms, as a suction lower than −1 MPa (pF 4.5) is needed to extract the first two layers of water from Ca-montmorillonite (see Figure 7).
3. The interparticle porosity remains saturated under most hydric conditions in soil (see Figure 7). Upon desiccation, there is a continuous loss of water that corresponds to the collapse of these pores and in an equivalent loss of the apparent volume of the clay. These pores are highly deformable.
4. Hysteresis occurs on rehydration of Ca-smectites, as the result of the aggregation of quasicrystals during desiccation [12]. Sodium-smectites, which are widely used in laboratory experiments, are very infrequent in natural soils, whereas Ca-smectites are widespread.

Organic Constituents. Because microorganisms grow primarily within or in the vicinity of the organic or organomineral phases, which provide many of their basic nutrients as well as energy, some chemical and physical properties of organic constituents will be discussed.

Plant residues, present in the >50-μm fraction, are relatively inert chemically. They are characterized physically by high porosity, which is unsaturated at most water potentials, and they have an elastic behavior upon rehydration (Figure 8). On the other hand, the small-sized fractions of OM are composed of the small debris of

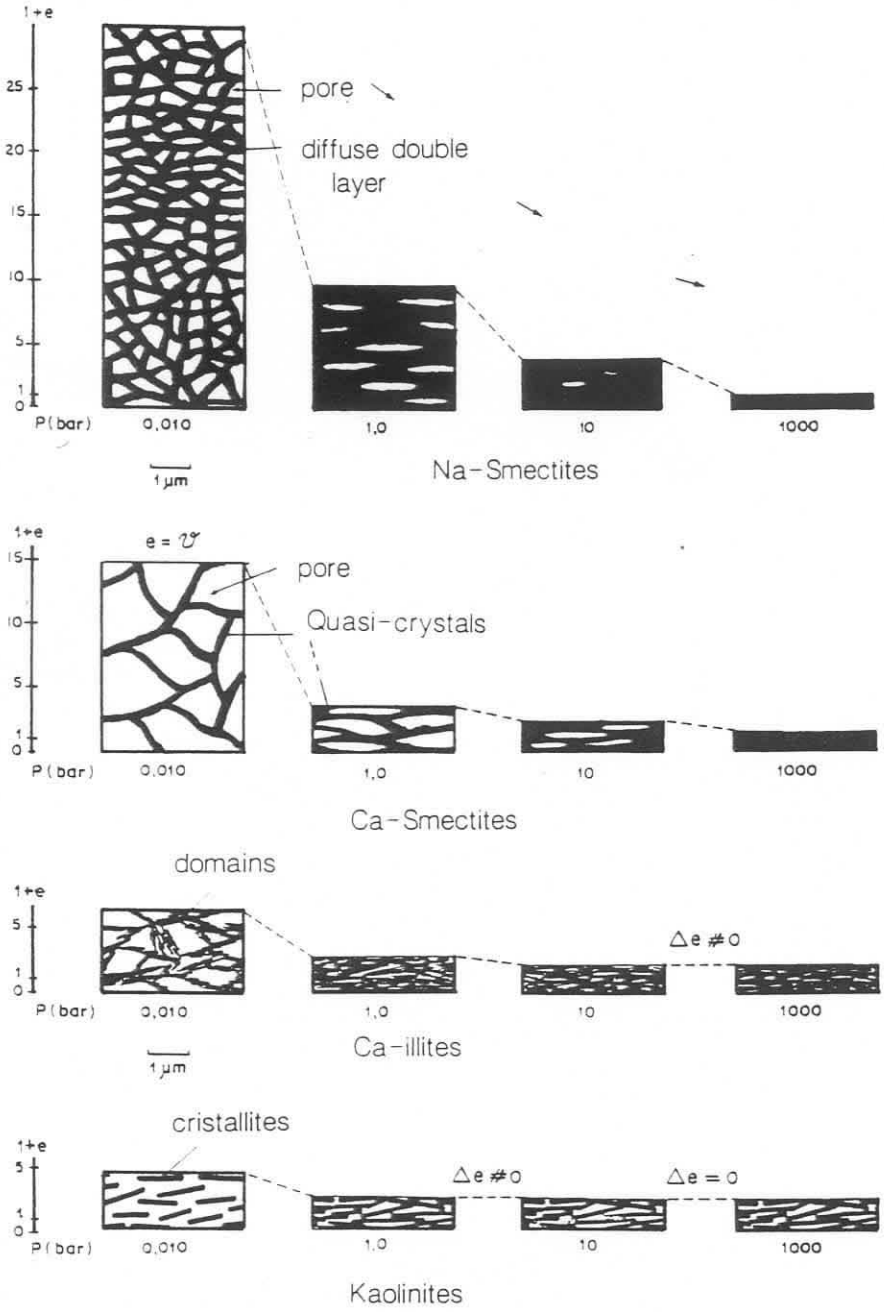


Figure 6 Variation in clay microorganizations and porosities with matric potential. e is the void ratio and ν is the water ratio (in $cm^3\ cm^{-3}$). From Ref. 12.

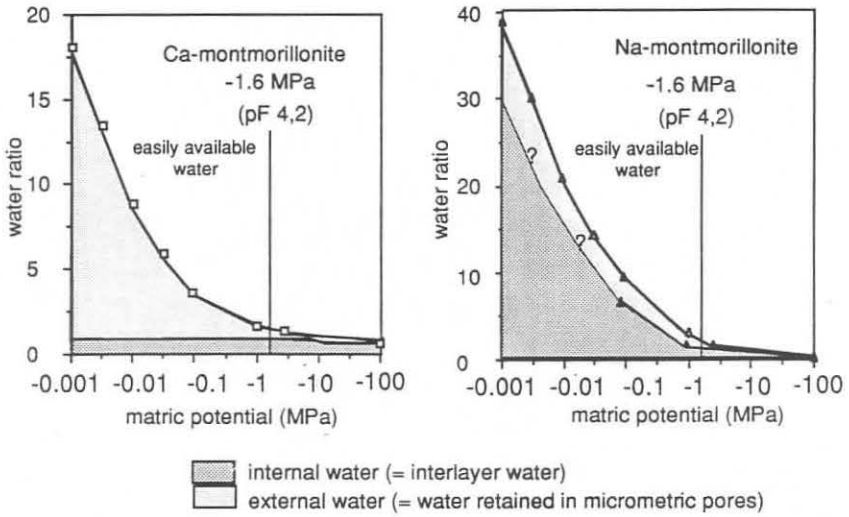


Figure 7 Water retention of Ca- and Na-montmorillonite in relation to matric potential. Water ratios (υ) are in $cm^3\ cm^{-3}$. Adapted from Ref. 12.

plants and microbes at different stages of decomposition. Most small-sized fractions of OM are amorphous and consist of humic materials or chemically defined polymers, such as polysaccharides or proteins, which may be particulate or soluble. Some organic compounds, such as polysaccharides, have high water retention capacities, generally with considerable hysteresis upon rehydration (see Figure 8). Knowledge about the structure and organization of humic compounds has advanced much less than that of clays. The initial configuration of OM was thought to be spherical, with a radius of 6–8 nm and a mean molecular weight of 80,000. However, such particles were seen only under vacuum and an electron beam. More recent studies have led to a concept of linear polymers that exist in solution as random coils, which can be more-or-less tightly coiled, and result in ellipsoid particles that are cross-linked or swollen, depending on the ionic strength, pH, and charge-compensating cations [42,43].

B. Associations of Constituents: The Aggregate Level

The most widely accepted definition of a *soil aggregate* is that proposed by Martin et al. [44], who defined it as "a naturally occurring cluster or group of soil particles in which the forces holding the particles together are much stronger than the forces between

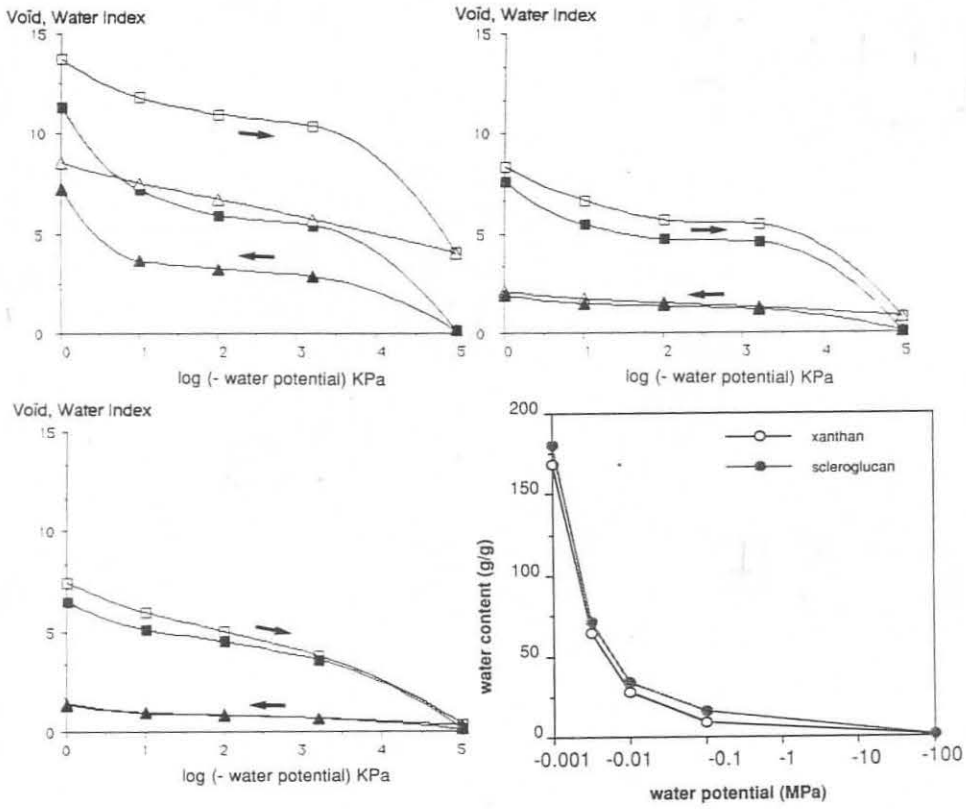


Figure 8 Water retention properties of different kinds of organic matter: (a) sphagnum peat; (b) herbaceous peat; (c) very decomposed wood peat; (d) bacterial and fungal extracellular polysaccharides. Symbols in a, b, c stand for: void ratios (□) and water ratios (△) in desiccation, and void ratios (■) and water ratios (▲) in rehydration. (a, b, c) From Valat, B., C. Jouany, and L. M. Rivière. 1991. Characterization of the wetting properties of air dried peats. Soil Sci., in press; (d) adapted from Ref. 124 and C. Chenu, unpublished data.

adjacent aggregates." Such aggregates are the basic units of soil structure; when they do not exist, structure results only from the juxtaposition of individual constituents. Between aggregates or constituents are pores, the volume of which depends on the size, shape, and stability of the aggregates or constituents.

Study of Soil Structure

Three main methods can be used to study the structure and aggregation of soil: (1) soil morphology at different scales; (2) fractionation of soil aggregates and indirect assessment of their stability; and (3) measurements of soil porosity. These methods can also be used to locate microbes and characterize their microhabitats in soil.

Direct Study of Soil Morphology. Soil macroorganization, with horizontal (soil horizons) or vertical zonation (prisms, fissures, tongues), will not be discussed here. On more microscopic levels, micromorphological methods are relevant to the study of interactions between microorganisms and minerals. One of the main problems, already mentioned for soil constituents, is to preserve the natural organizations that correspond to the state of humidity that prevails in the natural soils. A dissecting microscope can be brought to the field to study fresh clods [45], as well as to assess the microbial activity in soil. The same authors noted the importance of seasonal fungal development in A_2 Podzol horizons.

For scanning electron microscopy (SEM; 10^{-8}–10^{-3} m), critical-point drying or cryofixation of the samples for low-temperature SEM (LTSEM) is of particular interest. The soil aggregates around fungal hyphae and bacterial colonies can be observed after cryofixation [46,47]. Light microscopy (10^{-3}–10^{-7} m) and transmission electron microscopy (TEM) (10^{-4}–10^{-10} m) can now be performed on thin sections of soils obtained without too much perturbation of the samples by the use of fixative and hardening agents, and staining can be performed afterward [48]. Light microscopy allows the direct visualization of aggregates, pores, and main soil constituents, including roots, hyphae, and bacterial colonies [49,50]. Some quantification of the porosity can be achieved with light microscopy (quantimetry) [51,52], and it is easier if pores have been made fluorescent under ultraviolet (UV) radiation by a special dye. Pore quantification is also possible with SEM using cathode luminescence and backscattered electrons [53]. Microaggregates, microorganisms, biological remnants, and even amorphous organic matter can be visualized with TEM (see Figure 4).

To visualize all the different levels of soil organization and pores, it is necessary to combine these different methods. For example, combined light microscopy and TEM studies may be used to characterize porosities from several millimeters to nanometers in size. These observations are in good correlation with other porosity measurements.

Darbyshire et al. [54] have recently applied such methods to characterizing the microenvironment of soil microorganisms in undisturbed samples. Both UV light microscopy and SEM with backscattered

electrons were used to estimate the soil pore network available to protozoa. Such a fluorescent technique was also used for viewing fungi in the presence of soil particles [55]. A new promising field of research seems open, but we must emphasize that, for all of these morphological studies, the biggest problem is not to disturb the organization of the soil from the sample collection for observation of the thin, or ultrathin, sections.

Fractionation of Aggregates. As indicated in Section I.A, the separation of aggregates implies the use of less destructive methods than those required for the separation of individual soil constituents. Physical fractionation in water allows stable tropical soil micropeds or "pseudosand" aggregates, which are composed of associations of clay particles with iron oxides or hydroxides, to be separated [43,56,57]. Organomineral aggregates resistant to dispersion are present in the 0.2- to 20-μm fraction of soils [6,7,58]. Conversely, methods of size fractionation are used to assess naturally occurring levels of organization in soils and their mechanical resistance [59,60].

Fractionation of soil aggregates and assessment of their stability classically involved wet sieving [61], simulated rainfall, and swelling and dispersion in electrolytes [62]. Ultrasonic dispersion methods were subsequently developed [63,64].

Methods for the fractionation of aggregates are also interesting in soil microbiology because they allow the differentiation and separation of microbial cells that are outside or inside the aggregates (e.g., the washing–sonification method of Hattori [2]). The main limitations are that (1) microorganisms adhering to the outside can stay on the aggregates upon washing, and (2) physical techniques are very often insufficient to separate biomass and metabolites from inorganic components. Such findings were illustrated with ATP determination by Ahmed and Oades [65], and by the fractionation of particle-attached and unattached bacteria [66].

Porosity Measurements. Porosity is one of the most important characteristics of both the physical properties of soil and the development and mobility of microorganisms. One of the major techniques to evaluate soil porosity is to measure apparent density, either in the field or in the laboratory, on an unperturbed sample. Such a measurement takes into account both the density of the constituents and all the voids in the sample.

On small clods, it is possible to measure, by the methods described in Section I.A, the volume of solid, air, and liquid at each water potential value (Figure 9). The Laplace law expresses the maximum diameter of water-saturated pores according to the suction pressure (Table 3).

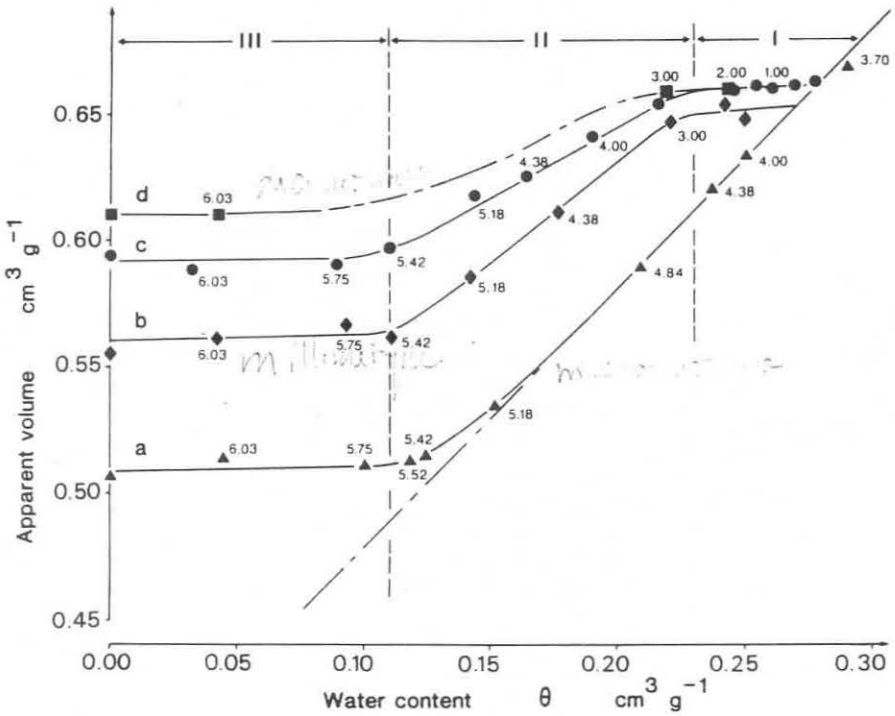


Figure 9 Shrinkage curves in a clayey soil (Terres d'Aubues) for samples of micrometric (a), millimetric (b), centrimetric (c), and decimetric (d) size. The areas separating the various curves correspond to the different levels of porosity, i.e., pores corresponding to the stacking of clay particles (below curve a), tubular pores (between curves a and b), and millimetric or centrimetric cracks (between curves b and d). Phase I correspond to the draining of pores with unchanged geometry, phase II to both shrinkage and draining of pores, and phase III to the shrinkage limit [68].

The Laplace law:

$$d = \frac{2\Gamma \cos \theta}{P \rho g}$$

where

d is the pore diameter
Γ is the surface tension of water
θ is the contact angle of water
P is the suction pressure
ρ is the volumetric mass of water
g is gravity

Table 3 Water Potential Expressed with Various Units and Maximum Size of Water-Saturated Pores According to Laplace Law. Low water potentials correspond to high pF and low water contents.

pF	Ψ (MPa)	Ψ (bars)	a_w	maximum diameter of water-saturated pores (μm)
1	-0.001	-0.01	0.999993	300
2	-0.01	-0.1	0.99993	30
3	-0.1	-1	0.9993	3
4	-1	-10	0.9927	0.3
5	-10	-100	0.927	0.03
6	-100	-1000	0.484	0.003

It is also possible to measure directly the pore volume and the equivalent pore radius using mercury or nitrogen penetration into the sample. The main problem with this method is that the sample has to be air-dried or lyophilized. If the soil structure is stable enough after drying, these techniques can provide interesting data that are relevant to microorganisms in soil, such as total pore volume, and the distinction between intra-aggregate (interparticle) porosity (which is often in the range of a few nanometers) and interaggregate (Figure 10) porosity (which ranges from a few to more than 10 μm). The range of porosity can be defined from mega-, through macro- and micro-, to nanoporosity.

The size and arrangement of individual particles, or the presence of aggregates, determine the retention and circulation of water in soil. If only texture is considered, clayey soils have mainly hydric properties that characterize pure clay minerals (e.g., high water retention and large variations of apparent volume upon desiccation and rehydration). However, differences exist between smectitic and kaolinitic soils (Figure 11) [67]. In contrast, sandy soils have low water reserves, and there is no change in apparent volume during gain or loss of water (see Figure 11). Silty soils have intermediate properties. General relationships between soil texture and porosity (amount and size of the pores) exist.

Each kind of porosity has its specific function (e.g., drainage of excess water and aeration for big pores, and retention of water with higher and higher energy when the pore radius is decreasing [68]). Recent emphasis has focused on the importance of such

determinations for microorganisms, for example, water-filled pore space (WFPS) has been demonstrated to regulate microbial activity, especially relative aerobic versus anaerobic processes [69–71].

Examples of Aggregated Soils

By using the techniques described in Section I.B, it has been demonstrated that many soils have specific aggregation levels, which are related to their constituents and conditions of genesis. For example, in brown acid soils, two different types of microaggregates (<250 μm) were recognized: the first was the result of microbial activity (see Section IV.B), whereas the second was related to chemical Al coatings on the clay [72]. In mollisols, the formation of stable microaggregates in the range of 0–20 μm is probably caused by organic matter [43]. The nature of the clay minerals also appears to have a role [e.g., larger aggregates (> 250 μm) are formed with OM when montmorillonite is the dominant clay].

In vertisols, clay aggregation occurs only in the subsurface. In the profile, the main levels of porosities are the micrometric pores of the smectite quasicrystal network, together with fissures formed upon drying and some biological pores resulting from roots and fungi (Cabidoche, 1990, personal communication). Both types of porosities are revealed bimodally by water retention curves (see Figure 11). In these soils, bacteria were demonstrated to live mostly in the micrometer-scale pores [73], rather than on the walls of the fissures [59].

In clayey tropical soils, two very different structures were distinguished [56,74]. Red tropical soils are well structured: clay particles are linked together into 100- to 500-μm aggregates by Fe and Al cements (see Figure 10). In yellow soils (i.e., degraded red tropical soils), soil aggregation is absent, and clay particles occur as individuals. Porosities are different in the two types of soil and result in different hydric properties.

In andosols, two main scales of organization have also been shown: nanoaggregates (1–10 nm) resulting from the association of elementary mineral particles of allophanes, imogolites, or halloysites (which are very small: 1–10 nm); and microaggregates (100–1000 nm) resulting from drying or faunal activity [75].

In addition, the soil management or action of macrofauna can result in the formation of microaggregates and clods which are loose associations of microaggregates or individual constituents, with larger pores between them [58,60]. All these levels of organization are of great importance for both soil properties and microbial life.

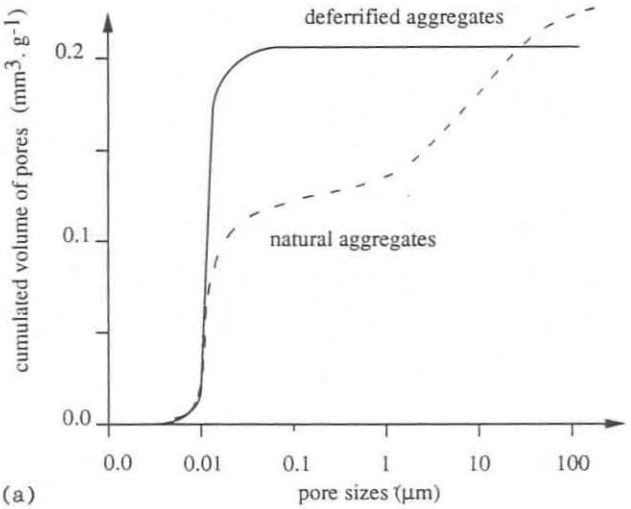
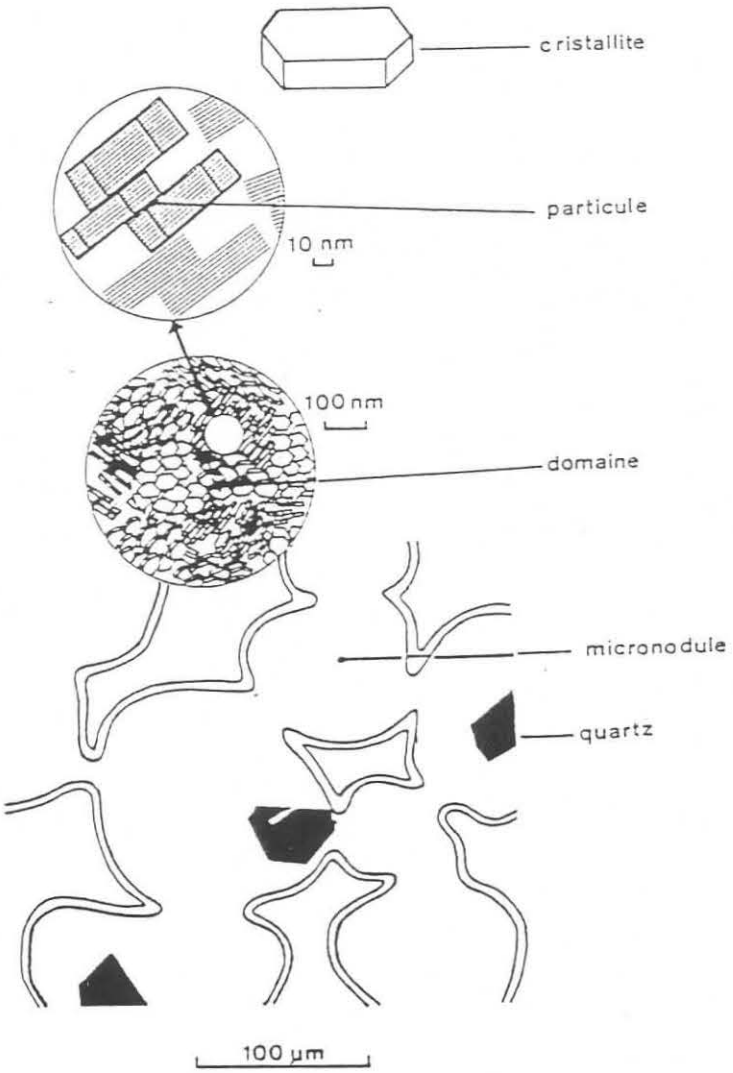


Figure 10 Levels of aggregation in an Oxisol: (a) porosity curves for natural aggregates and deferrified aggregates: absence of a 10-nm aggregated level; (b) schematic representation of the different levels of organization [57].

Conclusion. Some important properties of soil constituents have been discussed. The chemical properties have certainly received the most attention relative to microbial functions [1]. These chemical properties are directly related to the colloidal level, especially to the type of layer and interlayer of the clay minerals and to the kind of primary structure for OM. However, it is also necessary to consider the importance of the interparticle surface area of clay minerals and the extremely small size of clay particles in soils; as well as variable charges, which exist in half of the soils of the world, relative to coatings and low-range crystalline compounds [76].

The physical properties of soil that can affect microbes have been considered less than the chemical properties for various reasons. Soil physical properties invoive the tertiary and quaternary structure of OM and the various levels of soil organization, from the colloidal to the aggregate level. Until recently, clay and soil organizations were not well understood. However, the development of new methods, based on morphological studies at different scales, have helped solve this problem. Moreover, until recently, physical constraints were more difficult to control and to experiment with than were chemical constraints. Another reason is the difficulty


cristallite
particule
10 nm
100 nm
domaine
micronodule
quartz
100 µm

(b)

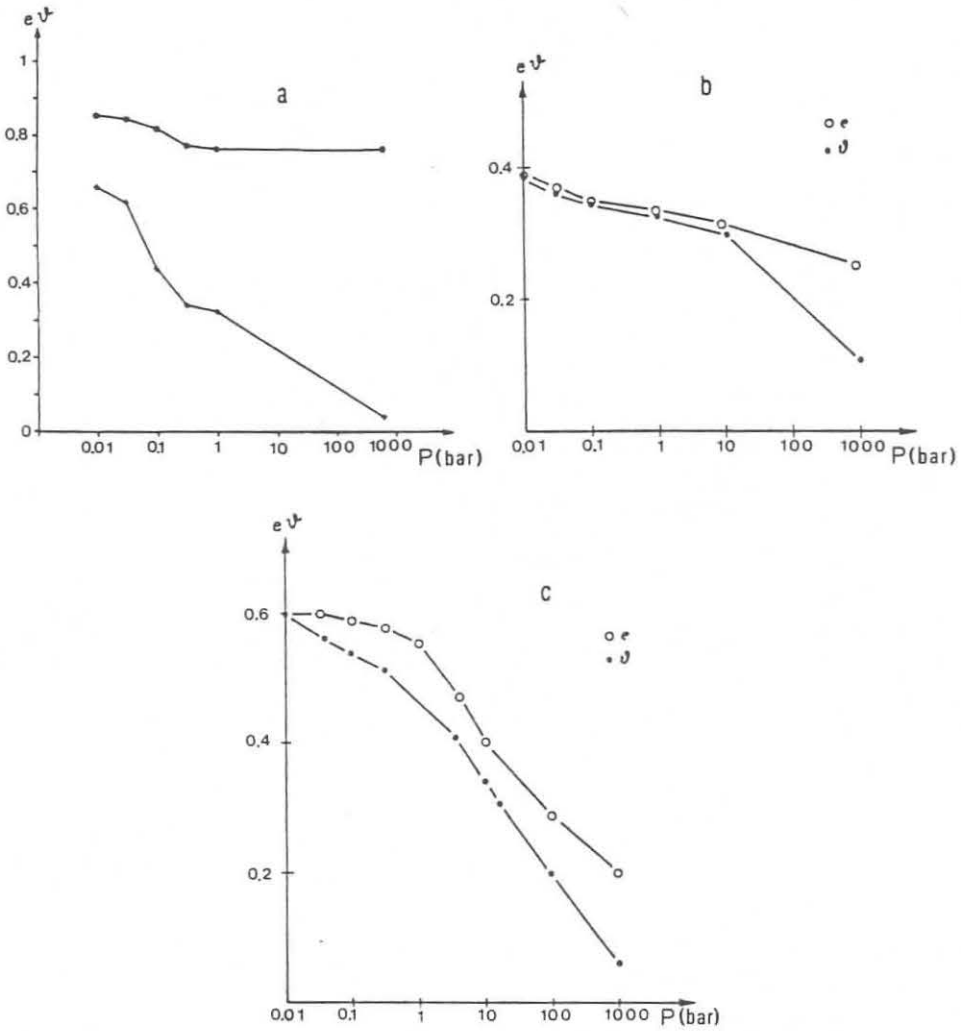


Figure 11 Shrinkage curves in soils of sandy (a), loamy (b), or clayey texture (c). ν is the water ratio and e the void ratio (both are in $cm^3\ cm^{-3}$ [67].

in locating and quantifying accurately microorganisms in soil samples.

In the second part, some data on interactions between soil minerals and microbes, as influenced by these properties will be presented.

III. INFLUENCE OF THE MINERAL ENVIRONMENT ON MICROORGANISMS

Most of the research on the effects of soil minerals on the activity of microbes has been conducted at the colloidal level (i.e., the effect of clay minerals on microbes) rather than at the aggregate level.

Microbial functions, such as respiration, growth, reproduction, spore germination, nutrient uptake, can be affected by clay minerals (see [1], for a comprehensive review). The effects of clay minerals, particularly of the most active ones (i.e., smectites) appear to be very important and very complex [1,77–80]. For example, the addition of small amounts of clay, especially of montmorillonite, stimulates the respiration of fungi [81]. Further increasing the amount of clay (>4%) gives the reverse effect. The first phenomenon was explained by a chemical action (i.e., buffering the pH of the medium), whereas the second one was mainly physical (i.e., the result of increased viscosity and reduced O_2 diffusion into the medium). The latter phenomenon can be explained by a better understanding of smectite organization (see Section II.A).

Adhesion of the microorganisms on clay minerals has been hypothesized to modify several aspects of their biological functions, although most effects of clay minerals on the activity of microorganisms do not necessarily imply adhesion [1]. More detailed aspects of the consequences of adhesion on microbial activity will not be discussed here, as comprehensive and recent reviews discuss the topic [e.g., 1].

The general effect of clays on microorganisms involves chemical aspects (e.g., the adsorption properties, charge, cation-exchange capacity, and cation retention by clays) as well as physical aspects (e.g., water retention, porosity, viscosity, and diffusion of O_2). The effects of clay on microorganisms have been studied mostly in pure liquid cultures [1,82]. Such an approach emphasizes the chemical effects. It should also be noted that in these suspension conditions, the clay surfaces are highly accessible to microorganisms or organic materials, whereas accessibility is probably lower in soils. This might be very important for interactions in which the clay minerals are involved as adsorbents. It is likely that in soils the physical effects of clay minerals will, in many situations, be as important as, or even overwhelm, the chemical effects.

Several actions of the soil mineral constituents on microbial activities occur at the aggregate level. Soil minerals and their organization influence, for example, the availability of water and the water potential conditions in which microbes live, the diffusion of oxygen and the relative occurrence of aerobic versus anaerobic

conditions, and the accessibility of organic substrates for microbes to feed on.

An example of a major physical effect of soil minerals is how soil constituents and their organization affect microbial activities through water potential. Chemical factors will then be considered with the example of various elements that can cause major stresses for microbes: iron, through its availability to microorganisms and aluminum by its toxicity.

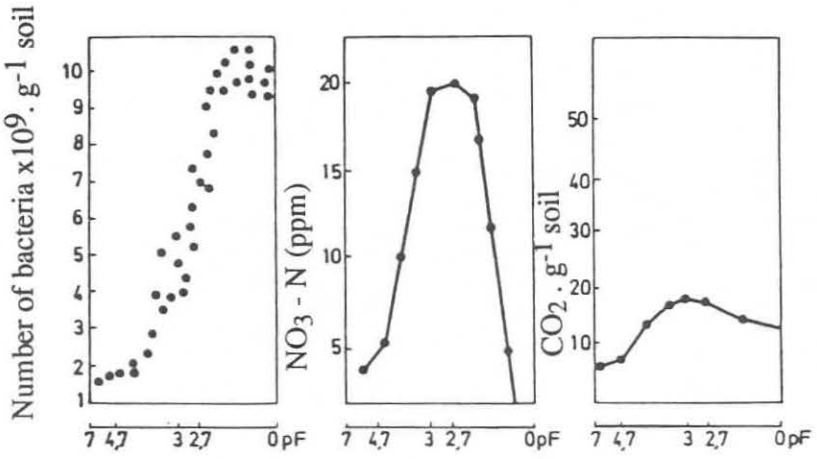
A. Physical Stress in Relation to Soil Minerals: Water Potential

Soils are porous media with variable water contents and water status. The most important physical aspect of microbial ecology in soils is related to the water potential. It is one of the main factors that governs both survival and activity of microorganisms and the competition between them [83,84]. The definition of water potential and its role in clay and soil organization were presented in the first part of this chapter. In most soils, the salinity remains lower than 0.2 g L^{-1} and thus, the matric potential is the major component of the soil water potential, and this discussion will mainly focus on it. However, with the development of irrigated agriculture, soil salinity has become an ever-increasing problem, and salinities as high as 1.5–3 g L^{-1} can be measured [85]. Hence, more knowledge of the sensitivity of microbial populations, especially root nodule microbes, to osmotic stress is needed. However, it is frequently accepted that there is a good correlation between the effect of matric and osmotic potential [86–88].

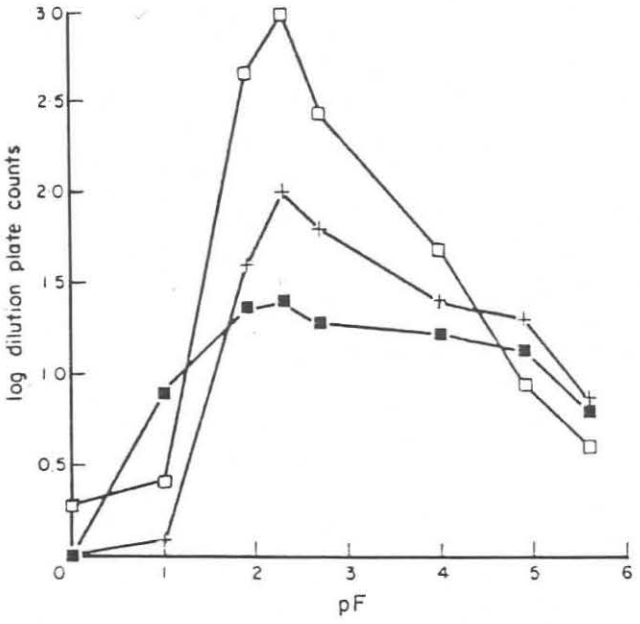
In this review, the direct effects of variations in water potential on the activity of microbes are considered first. However, most aspects in which soil minerals are involved in the activity of microbes usually involve indirect effects of the clays on water potential.

General Effects of Water Potential

Disregarding, for the moment, very high water potentials, i.e., high water content, high water activities a_w (Table 3), there is a general correlation between water potential and the number and activity of microbes, such as numbers of bacteria (Figure 12) [89], hyphal growth [87,90], and respiration [83]. Maximum rates of microbial respiration, nitrification, and mineralization occur at the highest water content (lowest Ψ) at which soil aeration remains nonlimiting [91,92]. For many soils, this corresponds to −0.3 to −0.01 MPa, that is, to water contents of 10–35%, or 60% of the water-holding capacity, of the soil (Figure 13) [70,92,93]. Within the range of −0.03 to −1.5 MPa, the activity decreases linearly with the water potential [92], and the activity of microorganisms becomes very



a



b

Figure 12 Dependence of the number of microbes and activities on water potential (a) number of bacteria, nitrification, and CO_2 production in a calcareous soil [89]. (b) Numbers of actinomycetes (+), bacteria (□) and fungi (■) in a Al soil horizon [90].

much less below −10 MPa (pF 5) (Figures 12 and 13). Dommergues [94] found a water potential limit for microbial activities at about −40 MPa (pF 5.6). Soil water potential conditions usually range from saturation to −4.5 MPa in the upper meter of cultivated soils [39].

The effect of water potential differs widely with the different kinds of microorganisms. It has been established for decades that bacteria are more sensitive to low water potentials than are actinomycetes or fungi (see Figure 12). Most fungi can grow without problem above −1.5 MPa [95], and in this range of water potential, the fungi have a competitive advantage over bacteria when the stress is increased. Independent of these general trends, each microbe, especially each fungal species, has its own optimum and minimum water potential for growth [84]. Thus, the matric potential can determine some general ecological distributions, for example, of soilborne pathogens, which can grow in dry (e.g., *Fusarium graminearum culmorum* and *F. roseum graminis*) or wet soils (e.g., *Gaeumannomyces graminis*, various species of *Pythium*). These pathogenic fungi have higher resistance to dryness than do their plant hosts. Changes in water potential can confer a relative competitive advantage to some microorganisms [96], which provides the possibility of biological control. For example, *F. roseum* has a relative advantage below −1 MPa (pF >4) when bacteria that can lyse their hyphae are absent.

Direct Effect of Water Potential on Microbial Cells

The internal water potential of microorganisms is in equilibrium with the outside soil water potential [97]. Passive equilibration of the cell potential can occur through cellular plasmolysis and a decrease in internal water potential. However, this does not allow the physiological activities to function and the cell to survive. Many microorganisms can actively decrease their internal water potential by accumulating solutes in response to external decreases in either the matric or osmotic potential. This topic has received considerable recent attention [98,99], advancing to the level of genetic control [99,100]. The production of osmoprotectant solutes seems to be general among soil microbes [97]. Microbes devoid of any osmoprotectant solutes would not be able to survive water potential stresses, with decreases in water potential being immediately followed by cell plasmolysis and dehydration.

Osmoregulation. Osmoprotectant solutes can be more-or-less compatible with physiological activities; for example, compatible solutes in procaryotes are, in general, amino acids and quaternary ammonium compounds. Gram-negative bacteria (e.g., *Pseudomonas aeruginosa*;

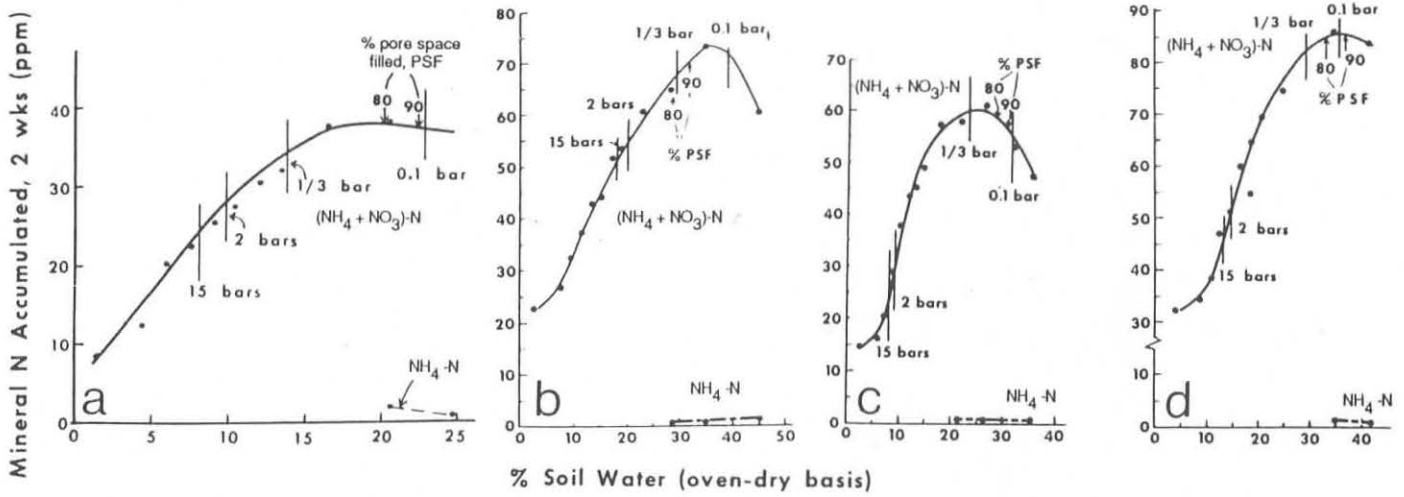


Figure 13 Mineral N accumulated in soils of various texture in relation to soil water content, matric potential, and percentage of water-filled pore space. (a) Sandy loam, (b) silty, (c) loamy, and (d) clayey soils. From Ref. 92.

rhizobia) accumulate potassium glutamate [98,101], and proline and aminobutyrate can also be accumulated (e.g., in species of *Serratia* or *Klebsiella* [98,102]). Gram-positive bacteria tend more to accumulate neutral amino acids, such as proline [98,102]. Actinomycetes such as streptomycetes accumulate proline, alanine, and glutamine [103]. Eucaryotic fungi and algae accumulate sugars and polyols, such as glucose and glycerol [104–108], trehalose [109], or sucrose [110].

The osmoprotectant solutes can be either constitutive (i.e., normally accumulated by the cells) or inducible (i.e., their production can be induced under water potential stress) [97]. Some microorganisms accumulate constitutive solutes only, such as potassium glutamate, in procaryotes, and polyols, such as arabitol or manitol, in eucaryotes. When unstressed, the cells of such microbes have high internal turgor, and consequently, their walls are very strong to withstand this pressure. If the magnitude of the decrease in water potential is less than the "buffering" capacity provided by the constitutive solutes, these microorganisms are not affected. For example, the growth of the gram-positive bacteria *Arthrobacter crystallopoites*, was not affected when the $\Psi > -1.5$ MPa in relation to glutamate accumulation [111]. Beyond this threshold the cell plasmolyzed, and such microorganisms are not well suited to soil habitats that are characterized by continual variations in water potential, and they are not very abundant in soils [97].

Most soil microorganisms can produce either inducible solutes (e.g., most gram-negative bacteria), or inducible plus constitutive solutes (e.g., gram-positive bacteria, which have thick walls) [98]. Eucaryotes producing both constitutive and inducible solutes are species adapted to environments with constant low water potentials (e.g., Xero-tolerant strains of *Saccharomyces* or halophilic algae such as *Dunaliella viridis*) [104].

In soil, osmotic stresses are mainly related to permeating solutes, which must be taken into account in laboratory studies of osmoregulation of soil microbes, as the physiological responses can be different from that in soil. For example, Busse and Bottomley [112] showed that *Rhizobium meliloti* strains induced the formation of glycine betaine and K^+ formation when low Ψ was caused by permeating NaCl but not with a nonpermeating solute such as polyethylene glycol.

Physiological responses of microbes can also differ according to the magnitude of the water potential stress. For example *Streptomyces griseus* and *S. californicus* were shown to induce the production of amino acids upon osmotic stress. Beyond a threshold of 0.75 M NaCl there was little further accumulation of amino acids, but intracellular K^+ increased [103]. It was hypothesized that the accumulated amino acids reached their limit of solubility in the cell or that a higher concentration could be inhibitory to enzyme function.

The desiccation process in soil is, in general, slow enough to allow the microorganisms to accumulate osmoprotectants. On the contrary, as discussed by Kieft et al. [113], the most rapid changes in water potential occur when a dry soil is wetted. The difference between the soil Ψ and the higher internal Ψ of the microbe causes an influx of water into the organism. This influx of water results in high turgor pressure, release of internal solutes, and can lead to cell lysis (plasmoptosis) and bursting of the cell wall [106,114]. Factors of resistance to dilution shock include the thickness and cell wall and the ability to release or polymerize internal solutes without a loss in viability, such as glycerol in lichen [105] or sugars in the alga *Rivularia atra* [109].

Forms of Resistance to Drying. One of the main ecological advantages of fungi and actinomycetes is their ability to persist as dormant propagules, such as sclerotia, oospores, or chlamydospores [115]. These forms are of particular importance to soil-borne plant pathogens, as they have great resistance to extreme desiccation for long periods and can give rise to hyphae and other organs of reproduction in the presence of hosts or in appropriate moisture conditions.

The effects of water potential on reproduction are complex and depend on which step of the reproduction cycle is affected [87]. Spore germination can be less sensitive to low water potential than growth, whereas sporulation is more sensitive than growth (e.g., in *F. roseum*) [116], and Ψ values corresponding to soil saturation are needed for the discharge of zoospores. Hence, to better understand the competition between microorganisms in soil, knowledge of their resistance to low water potentials during all steps of reproduction, growth, and survival is necessary.

Role of Extracellular Polysaccharides. It is frequently suggested that extracellular polysaccharides of soil bacteria may efficiently protect them from desiccation [117,118]. Extracellular polysaccharides have been hypothesized, because they are very hygroscopic, to provide a source of water under dry environmental conditions [117] and to reduce the rates of drying and wetting of the microbial cell, thereby facilitating the rehydration of the cells without deplasmolysis [119,120]. However, the presumed beneficial effects of extracellular polysaccharides on desiccation lack convincing experimental evidence, and this concept needs careful evaluation.

The concept of protection against desiccation by polysaccharides comes mostly from microscopic observations of bacteria in soils: soil bacteria are frequently surrounded by a capsule or a loose slime that is polysaccharide in nature [121,122]. In soil aggregates subjected to one—six months of severe desiccation, gram-negative bacteria

were observed to be embedded in a thick layer of polysaccharides [123]. The cells had well-preserved cell structures, indicating the viability of the bacteria. The authors concluded that extracellular polysaccharides were a major factor in the survival of gram-negative bacteria subjected to desiccation. However, no attempt was made, in this study, to correlate the presence of extracellular polysaccharide with the preservation of cell structures.

The assumption of a protective role for polysaccharides is also based on the hygroscopic properties of many microbial polysaccharides [117]. However, the water retention curve of microbial extracellular polysaccharides indicates that high water retention is restricted to high water potentials and not to low water potentials (e.g., <-0.1 MPa) (see Figure 8) [124].

Pena-Cabriales and Alexander [125] observed that the addition of purified exopolysaccharides to a *Rhizobium* strain in soil samples allowed somewhat better survival upon desiccation. However, in all other studies in which comparisons of the survival upon drying in soils of mucoid (i.e., polysaccharide-producing) versus nonmucoid strains of bacteria, were reported (e.g., *Klebsiella aerogenes* [126], *Pseudomonas solanacearum* [127], and various rhizobia [125,128–131], no significant difference could be recorded between the polysaccharide-producing or nonproducing strains. Thus, the weight of the evidence is against a protective role for polysaccharides. On the other other hand, in support for a possible protective role for polysaccharides, it may be noted that (1) when a pseudomonad isolated from soils undergoing severe desiccation was subjected to high water potentials, the production of its extracellular polysaccharide was enhanced [132]. (2) Polysaccharide gels are used as inoculant carriers for microorganisms; the desiccation of the gel causes severe mortality among the microorganisms, but the survival remains better than without any embedding agent [133–136].

If they are not involved in resistance to drying, what is the role of extracellular polysaccharides? They must have some survival advantages, as it is unlikely that, under highly competitive and oligotrophic conditions, bacteria would produce functionless substances requiring substrate and energy for their synthesis. Other possible roles have been reviewed by Dudman [118] (e.g., storage functions, ionic barriers, virulence agents, protectants against predation, or adhesive agents). Some of these will be discussed later in this chapter (see Section IV.B).

Indirect Effects of Water Potential Involving Soil Minerals

High Water Potentials and Aeration. At very high water potentials, the matric and osmotic potential become negligible and the main factor is the excess of water, which fills all the pores, even those larger than 10 μm (see Section II.B; Table 3). Hence, gas diffusion is

reduced: in water the diffusion rate is about 1/10,000 of that in air [137]. For example, the diffusion coefficient of O_2 in air is 0.189 $cm^2\ sec^{-1}$ and 2.56 10^{-5} $cm^2\ sec^{-1}$ in water [138]. The low diffusion coefficients of O_2 become critical for aerobic microbial processes such as respiration, nitrification, and sulfur oxidation. For example, an optimum Ψ for nitrification is approximately −0.1 MPa [89], and water potential is directly or indirectly a limiting factor both at high (−0.0032−0 MPa) and low water potentials (below −1 MPa) (see Figures 12 and 13). Anaerobic conditions are characterized by the predominance of specific bacteria and specific chemical processes (e.g., production of CH_4, N_2, H_2, and H_2S).

The separation of anaerobic from aerobic conditions is assumed to correspond to Ψ values of −0.02 to −0.01 MPa [139] or to 70% of the water-holding capacity [69,140]. The threshold between aerobic and anaerobic conditions depends on the texture and structure of the soil, and neither the water content nor the water potential are particularly good indicators of aerobic versus anaerobic conditions [93] (see Figure 13). However, a meaningful factor to distinguish aerobic from anaerobic conditions is the proportion of soil pore space filled with water (water-filled pore space; WFPS) [70]. Aerobic microbial processes, such as respiration, increase linearly with the water content between 30 and 60% water-filled pore space, and declines beyond 60−70% (Figure 14) [70,71]. This relation is true for a wide range of soil textures. On the other hand, an anaerobic process, such as denitrification, occurs only beyond 70% WFPS [71,92,141]. The significance of an overall determination of the water-filled pore space when applied to microbial habitats depends on the organization and pore size distribution of the soil. Two of the soils considered by Doran et al. [71] exhibited microbial activity versus WFPS relations that were different from the other soils (i.e., their curves were shifted toward higher WFPS; see Figure 14). These soils were both weathered Hawaiian soils, with very fine granular structure, and it is probable that the active microbes were located in the interaggregate porosity, whereas the intra-aggregate pores, which were taken into account in the WFPS, were water-saturated and biologically inactive.

Clay Minerals and the Resistance of Microbes to Drying. With the purpose of inoculating soils with root nodule-forming bacteria, many studies have been conducted on the survival of rhizobia exposed to desiccation in soils, and clay minerals have been reported to protect bacteria from death associated with drying. The survival of rhizobia tended to increase with an increase in the clay content of the soil [142,143]. When clay minerals were added to sand or sandy soils, the survival of bacteria varied widely, depending on the clay type, amount added, and how the clay was added (as a dried powder

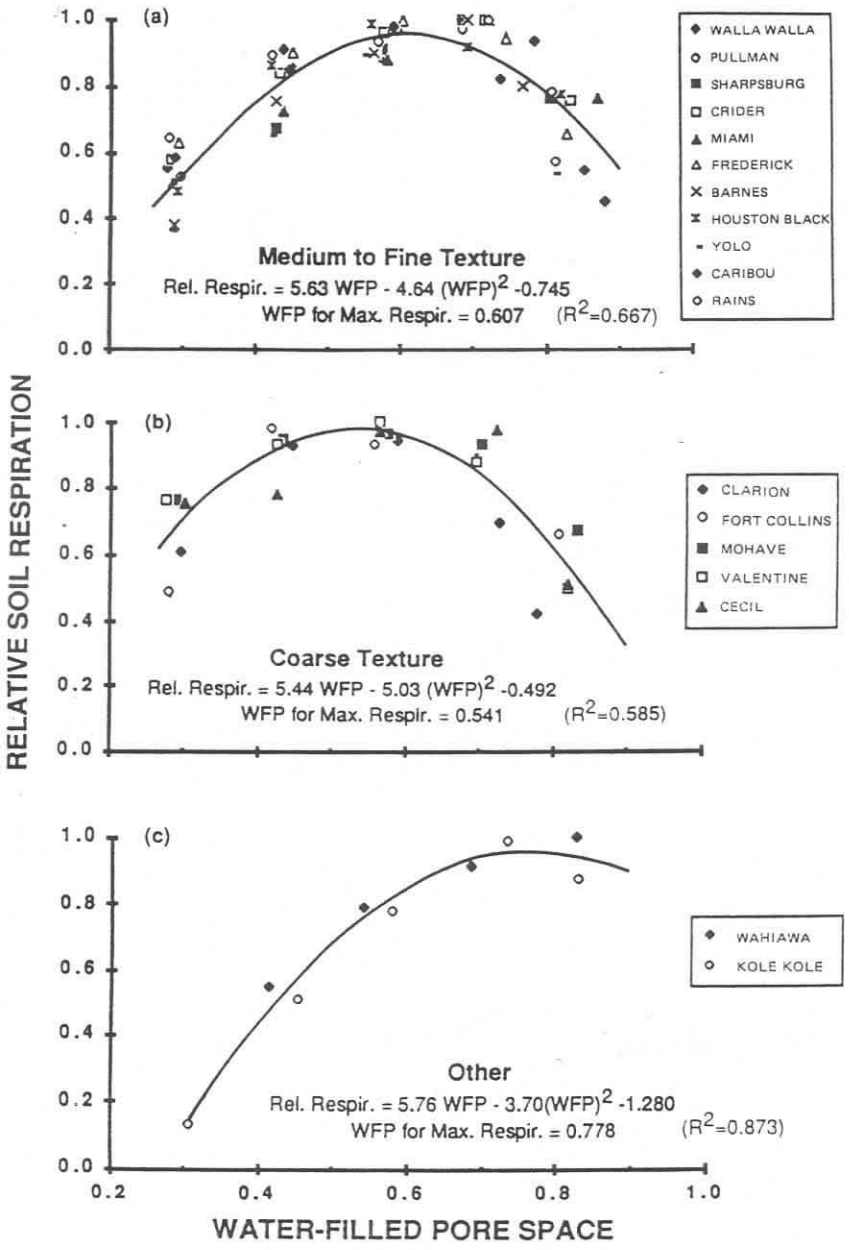


Figure 14 Relative soil respiration as a function of water-filled pore space (WFPS) for soils of various textures [71].

or as a suspension) [128,142]. Additions of kaolinite decreased the survival of *Rhizobium japonicum* in sand, whereas additions of illite had either no effect [142] or improved survival [17,131]. The addition of montmorillonite had, in general, a positive effect [126,128, 142], although no or negative effects were reported for some strains [128,142]. The protecting effect of clays was greater with strains less tolerant to desiccation [128,142].

A factor of major importance in these experiments was the rate of drying: for example, Bushby and Marshall [128] reported that the fast-growing rhizobia were more susceptible to desiccation than the slow-growing species under conditions of rapid drying. This result was confirmed by Jansen van Rensburg and Strijdom [144], although these authors found opposite patterns under slow desiccation: greater survival of fast-growing rhizobia than of slow-growing ones. They indicated that slow drying is probably more representative of drying conditions in the field than rapid drying, as in the Marshall procedure. The slower the soil dries, the greater the bacterial survival [145–148]. The protecting role of clay minerals might be to lower the rates of drying of the soil, which has been demonstrated [126,131]. The beneficial effect of clays can then be expected to depend on the rate of drying.

The numbers of survivors were directly related to the final water content of the soil matrix [126,142]. Clay was hypothesized to provide more available water to microbes upon desiccation [126], although other authors have considered, to the contrary, that the high affinity of montmorillonite for water caused the bacterial cells to be dried more thoroughly [142,144]. Indeed, for a wide range of microbial species, better viabilities are ensured at very low humidities, rather than at intermediate ones [118,133,142]. Bushby and Marshall [149] and Al-Rashidi et al. [143] proposed a similar hypothesis based on water adsorption isotherms of rhizobia: the more desiccation-sensitive strains were those of which freeze-dried cells retained more water.

With reference to the water retention properties of the different clay minerals (see Section II.A: Figures 6 and 7), the effectiveness in protecting microbes can be expected to be kaolinite < illite and interstratified clays < montmorillonite, which is consistent with most of the above-quoted results. It is worth noting that in some experiments, the clay minerals had a detrimental effect [142], which occurred when the clay was added as a suspension, rather than as a powder [128]. Thus, such a lethal effect of clay minerals occurs when the bacteria and clay can be expected to be the more intimately associated.

The same mechanisms were hypothesized to explain the protective effect of clay minerals and of extracellular polysaccharides on the death of microorganisms associated with drying (i.e., both have

very high affinities for water). In thin sections of soils, both are frequently associated; that is, bacteria or bacterial colonies are embedded in polysaccharides, which are surrounded by a layer of clay minerals [48,121,123] (Figure 15). As discussed by Kilbertus [150] this "encapsulation" might be of ecological significance for gram-negative bacteria that are devoid of forms of resistance such as spores.

The protective effect of clay minerals (see Figure 15) has found application in their use as inoculant carriers, either for root nodule bacteria [130] or for the biological control of soil pathogens by selected microorganisms [151–153].

Mechanical Effects Associated with Water Potential and Clays. Recent studies have pointed out a new mechanism by which water potential can act on microorganisms through minerals. *Pseudomonas solanacearum* is an important soil-borne plant pathogenic bacterium that causes severe wilt of solanaceous crops in subtropical and tropical areas. In the French West Indies (Guadelupe), vertisols were shown to be suppressive to the disease, whereas oxisols are conducive [154]. The suppressiveness seems to be related to the dominant clays in these soils (i.e., montmorillonite in vertisols, halloysite and kaolinite in oxisols). Schmit and Robert [127] studied the survival of a mucoid (exopolysaccharide-producing) and a nonmucoid strain of *P. solanacearum* in the presence or absence of pure clay minerals (i.e., montmorillonite or kaolinite). Clay slurries were mixed thoroughly with the bacterial suspensions to a ratio of 2×10^{11} bacteria per gram of dry clay. The water potential was accurately controlled using the methodology presented in Section I.A. [40]. In these tropical soils, the water potential generally fluctuates between −0.01 and −2.5 MPa, so the matric potential was maintained constant at either −0.01, −1, or −2.5 MPa, or alternated from −0.01 to −2.5 MPa, and the survival of *P. solanacearum* was monitored by periodic plate counts. At high water potential (−0.01 MPa), the populations of bacteria were maintained close to the initial level, but low water potential caused a rapid decline in both strains (Figure 16). A comparison with the reference samples of bacteria without clay indicated that desiccation of the bacterial cells could not entirely account for the mortality in the clay at low water potential. As observed with SEM and TEM, the bacteria were located in the micropores between clay particles (see Figure 15). Such pores are closed between −0.1 and −1.0 MPa [12]. It is, therefore, assumed that the mortality was due to mechanical stresses as the pores sizes decrease below a limit lethal for cells. Both montmorillonite and kaolinite had lethal effect on cells. Pores in the micron range are abundant in vertisols, and the mortality of *P. solanacearum* below −0.1 MPa was confirmed in vertisols and in purified clays from

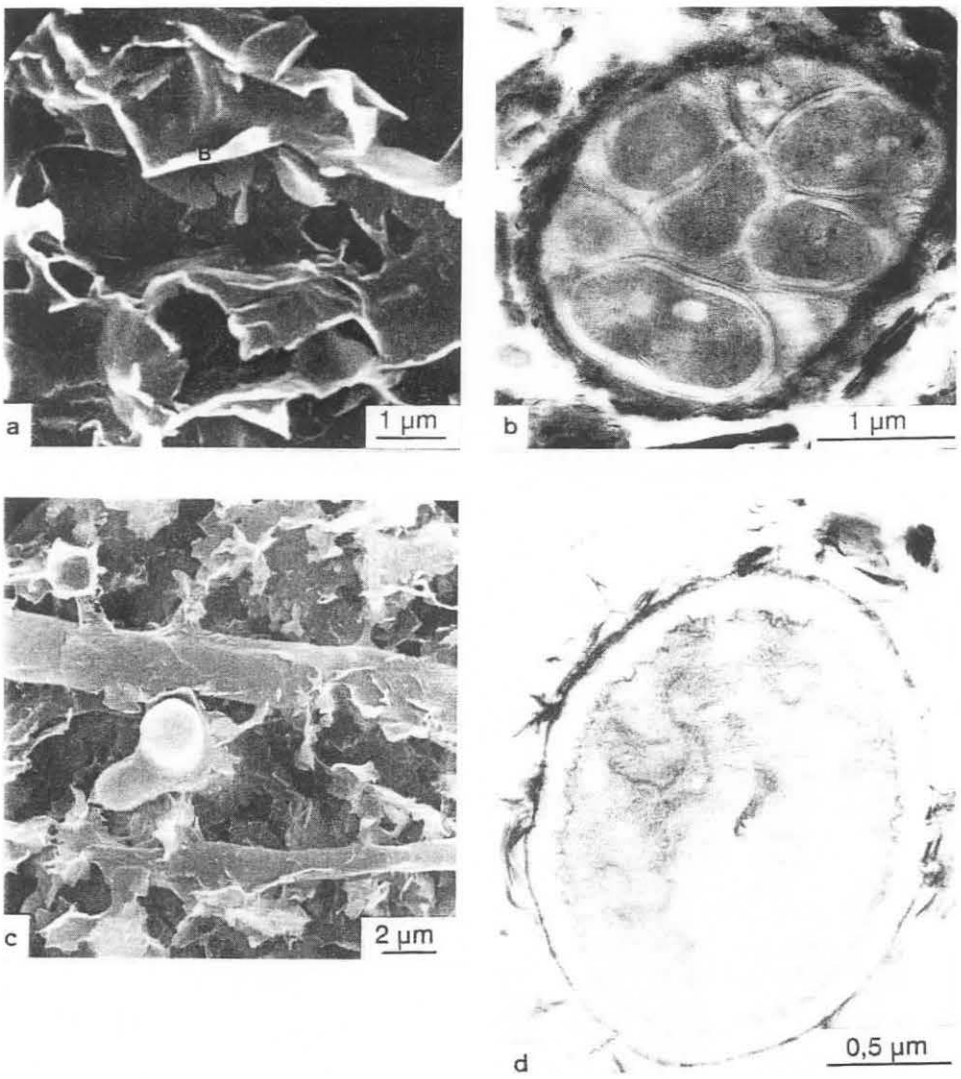


Figure 15 Soil microorganisms in their microhabitats: (a) bacteria (*P. solanacearum*) in the micrometric porosity in montmorillonite, low-temperature scanning electron microscopy [127]. (b) Bacterial microoaggregates in the 0.2- to 2-µm fraction of a soil, transmission electron microscopy observations on thin sections prefixed with OsO_4 and stained with uranyl acetate and lead citrate (C. Chenu, unpublished). (c) and (d) Fungi encapsulated in montmorillonite for biological control; (c) low-temperature scanning electron microscopy and (d) transmission electron microscopy. From J. Fargues, unpublished, and Ref. 152.

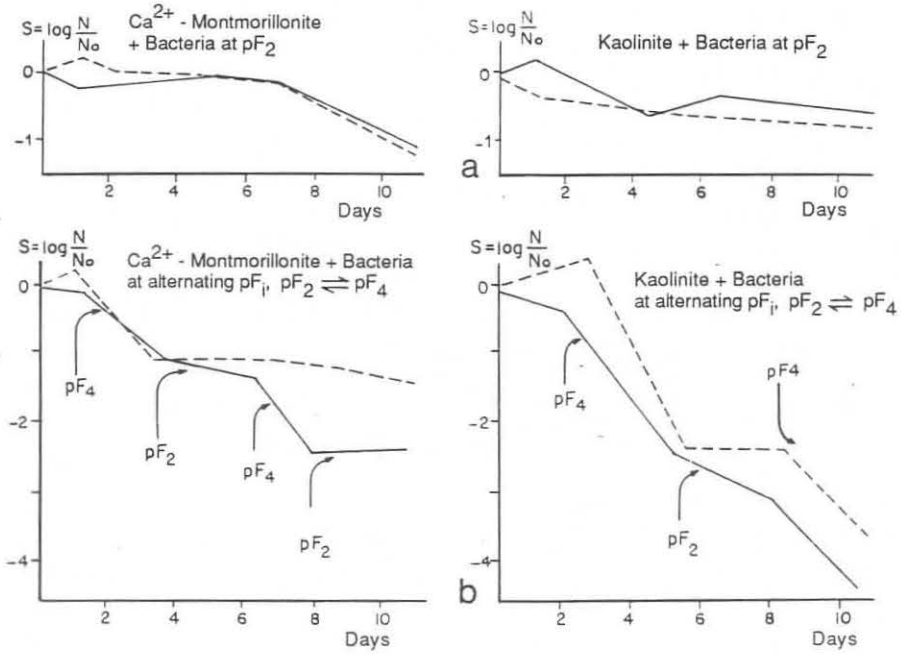
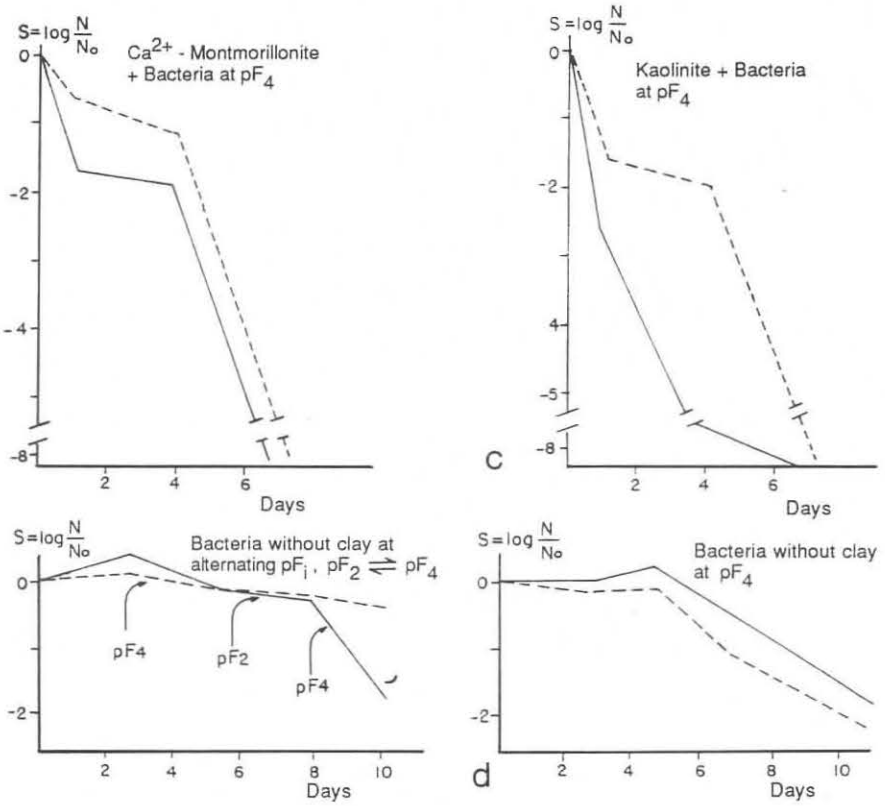


Figure 16 *Pseudomonas solanacearum* survival upon different water potential conditions in kaolinite and montmorillonite. Survival (S) is expressed as log N (final numbers of bacteria)/N_0 (initial numbers of bacteria). (a) The matric potential was maintained constant at pF2 (−0.01 MPa); (b) the matric potential was maintained constant at pF4 (−1 MPa); (c) the matric potential was alternating from pF2 to 4 (−0.01 to −1 MPa); (d) bacteria without clay, the matric potential was maintained constant at pF2 (−0.01 MPa) or alternating from pF 2 to 4 (−0.01 to −1 MPa) [127].

vertisols [73]. On the other hand, in oxisols, the kaolinite or halloysite particles are associated into stable micropeds with an interaggregate porosity larger than 2 μm (see Figure 10). In these pores, the bacteria could be subjected to a lack of water, but not to mechanical stresses, which may explain why oxisols are conducive to solanaceous wilt. Recently, irrigation of vertisols in some areas of Guadelupe greatly decreased the effect of the dry season and the disease has developed, giving field confirmation of the experiments of Schmit and Robert [127].



In some other cases of pathogenic microbes, soils were found to be suppressive or conducive, depending on their dominant clay mineralogy [155]. Biotic and abiotic factors are always involved in suppressiveness versus conduciveness. For fusarium wilts, montmorillonitic soils have been shown to be suppressive to the fungi by allowing the development of antagonistic bacteria for banana wilt [155] or for the muskmelon wilt [156]. Similar observations have been made by Stutz et al. [157] for the black root rot of tobacco and its antagonistic bacteria: the ability of the clays to support bacterial growth was vermiculite > montmorillonite > illite. Inasmuch as suppressiveness versus conduciveness occurred in the absence of severe desiccation, factors other than protection by clays against drying have to be considered, among them being mechanical factors. Chemical factors will be discussed in Section III.B.

Mobility of Microbes in Relation to Water Potential. It is generally assumed that there is no limitation to the movement of fungi and actinomycetes in soils as a result of their hyphal system [94]. Scanning electron microscopy observations by Dorioz and Robert [47] showed extensive colonization of clay pores by fungi at −0.01 MPa, although the pores in the various clay minerals were smaller than the average hyphal diameter. For other microorganisms, provided they have genetic capabilities for motility, their mobility depends both on the pore sizes and the volume of water in the pores. Both of these parameters depend on the nature of the mineral constituents and on the water potential. Most microbes require pores larger than their own size and that these pores be filled with water. Amobae and zoospores [115] require pores larger than 10 μm. Bacteria require proportionally smaller pores, between 1.5 and 2 μm, for movement [158]. The water potential necessary to fill these pores is above −0.1 MPa. How do bacteria move in soils? An undetermined point is whether bacterial strains that have flagella in pure culture also have them in soils [1]. Bacteria can also be expected to move passively, being carried by water flow after a rain or irrigation [126,159], by growing hyphae and roots, or by fauna [160]. Without "carriers," bacterial movements in soils would be very limited. Postma et al. [35] inoculated a silty sand and a loam silt with rhizobia and located the bacteria in pores of known size. This distribution persisted after an incubation of several weeks, indicating that there was no significant transport of the bacteria.

Soil Microhabitats in Relation to Water Potential. As discussed in Section II, soils are generally characterized by a complex spectrum of pores that depends on their level of aggregation. Hence, with alternating wet and dry conditions in the field, some pores are saturated and even O_2-limiting, whereas others undergo desiccation. In addition, the pores can be deformed upon desiccation, as seen in vertisols. Microhabitats with contrasting water characteristics exist in soils, as shown by the occurrence in the same soil of species with very different tolerances to desiccation or with very different requirements for O_2 (e.g., nitrification and denitrification frequently occur in the same soil at the same time).

Hattori [2,161] proposed a model for microbial ecology based on two main soil microhabitats: intra-aggregate microhabitats, characterized by small pores with available water under most soil moisture conditions; and interaggregate locations, subject to severe drying. Microbes on the outside of aggregates could easily be washed out of soils by gently shaking the aggregates in water, whereas sonication was required to disperse the microbes in inner positions. Differences in the distribution of microorganisms were shown; most fungi

are located on the outside of aggregates, whereas most bacteria occur within aggregates [2]. Gram-negative bacteria, which are very sensitive to desiccation, especially tend to be located inside aggregates [123,162], where they are presumably protected by being imbedded in extracellular polysaccharides and clay minerals [123].

The aggregate model of Hattori [2] was applied to aerobic versus anaerobic habitats and further developed by Klein and Thayer [163]. They differentiated three zones: One was aerobic on the outside of aggregates, another was aerobic with pores filled with both air and water in the outer part of the aggregate, and the last one was anaerobic and situated at the center of the aggregates and within pores filled with water. This model is certainly oversimplified, but it helps to understand organometal transformations (particularly with heavy metals that have various states of oxidation, such as Ag, Hg, Sn, Se, and Pb) [163]. Such a model can also be applied to denitrification: anaerobic microsites within soil aggregates have been invoked to explain the occurrence of denitrification in well-aerated soils. Direct demonstration was possible with O_2 microelectrodes, and anaerobic centers were shown within aggregates, provided that they were large enough (>4 mm) [164]. However, in the presence of very high consumption rates of O_2, as the result of the degradation of organic mater, "hot spots" of denitrification can occur, even when diffusion of O_2 is not significantly reduced [165]. Such a distribution of anaerobic versus aerobic microsites may exist even at a larger scale (e.g., in the prisms of the stable structures of vertisols, which can have considerable variations in the water content from the outside of the prism to the inside) [165].

Several comments can be made about the approach of Hattori [2]. First, the fractionation experiments are considerably affected by the structural stability of the aggregates. The distinction of inner versus outer zones implies that the aggregates are stable, which is probably not true for many soils. As noted by Jocteur Monrozier et al. [59], both inner and outer microniches could be differentiated in the A1 horizon of a vertisol, which was very stable, but only external microniches occurred in the very unstable A1 horizon of an alfisol. The partitioning of the microbes between inner and outer zones is presumably different among soils, depending on the levels of organization and structural stability, and the fractionation method should be adapted to each soil. In addition, several levels of aggregation exist in many soils, with polyaggregates composed of smaller and simpler microaggregates.

It is also necessary to know whether bacteria can enter aggregates that already exist in the soil. For example, in oxisols, the porosity between the micropeds (>10 μm) is accessible to microbes, but the internal porosity, which is smaller than 1 μm is too small

(see Figure 10). Similar features exist in the Bt horizon of alfisols [59].

Another approach is to characterize the microhabitats on the basis of the pore size. Kilbertus [150] demonstrated by TEM that most soil bacteria were located in 1- to 2-μm pores. Postma et al. [35] introduced *Rhizobium* into soils maintained at different water potentials. On the basis of different initial moisture contents, soil samples with water-filled pores of different diameters were prepared. It was hypothesized that upon further inoculation, bacteria would locate in pores not previously filled by water. After incubation at −0.01 MPa, the bacteria were washed out. It was observed that the initial contents of rhizobia retained by the soil decreased with a decrease in the initial moisture content of the samples: the less the porosity was initially saturated, the more places were vacant for inoculated bacteria. Retention of cells occurred when the matric potential was higher than pF 2 or 3 (Ψ less than −0.01 or −0.1 MPa). If the poral spectrum is assumed to be unaffected by wetting the sample during the inoculation, which is probably not true, it would correspond to pore sizes smaller than 9 or 0.6 μm. Micrometric pores, which are illustrated in Figures 3 and 15, would then represent protective microniches for introduced bacteria [35,159], and such microniches could be created artificially by incorporation of montmorillonite in the soil [166,167]. However, the protection seems to arise more from a physical protection from predation than from desiccation [168]. Several authors have demonstrated that inner habitats or small pores were not accessible to protozoans grazing on soil bacteria because of the small size of the pores [168–170]. Complex trophic interactions among nematodes, amobae, and bacteria were also related to pore sizes [171].

Thus, the poral spectrum of soils is of major importance for the possible habitats of microbes, as this determines hydric conditions, aeration conditions, trophic conditions, and relations between organisms (predation and competition) [172].

Conclusion

In conclusion, several points can be emphasized. The first is the importance of geometric constraints in soil for the activity of microbes. In particular, the sizes of the pores determine under which conditions of water potential they are filled with water and their accessibility to microbes or to predators. In addition, pores can be deformable, especially those within clay minerals. The second is the complexity of most effects of clays on microbial survival, as it is probable that several factors are involved at the same time, for example, protection against desiccation, availability of iron (see next Section IV.B), and competition or predation relations between soil organisms. Both physical determinations and morphological data

seem to be most important in elucidating these aspects of microbial ecology. The micrometric habitat of bacteria, which consists mainly of the interparticle porosity of clays, is of utmost importance. Such a microhabitat is quite complex: for example, when the clays are swollen under water-saturated conditions, the pores are open and allow circulation of bacteria. Under conditions of intermediate water potential the small size of the pores can protect bacteria from their predators. Under low water potentials, the pores can close, resulting in some lethal effects on microbes.

B. Chemical Stresses in Relation to Soil Minerals

Soils constituents are the main source of nutrients for microbes, and microbes obtain these elements (major or trace) through mineral weathering processes in which they participate.

Clay minerals have important chemical effects on microbes, mainly through their cation- or anion-exchange capacity [1]. Other mineral compounds, mostly associated with clay minerals, are of special importance for the microbial life: aluminum, which is toxic in acid conditions; iron, which is necessary for plant and microbes, but the availability of which is very low in certain neutral or alkaline soil conditions.

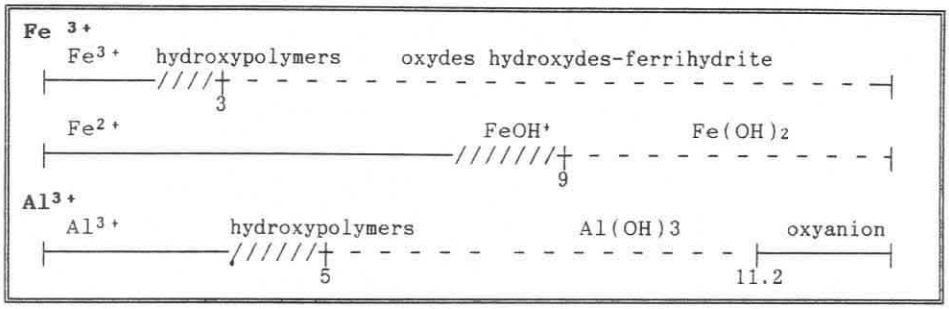
Effect of Iron on Microorganisms

This subject has received increasing interest in the last 10 years, and some authors have put forward the hypothesis that iron availability can govern competition between pathogenic fungi (e.g., *Fusarium oxysporum*) and certain bacterial species (e.g., *Pseudomonas fluorescens*). Although this subject is controversial and not completely clear, some elements will be discussed, both relative to iron speciation and the effect of iron on the suppressiveness of plant diseases.

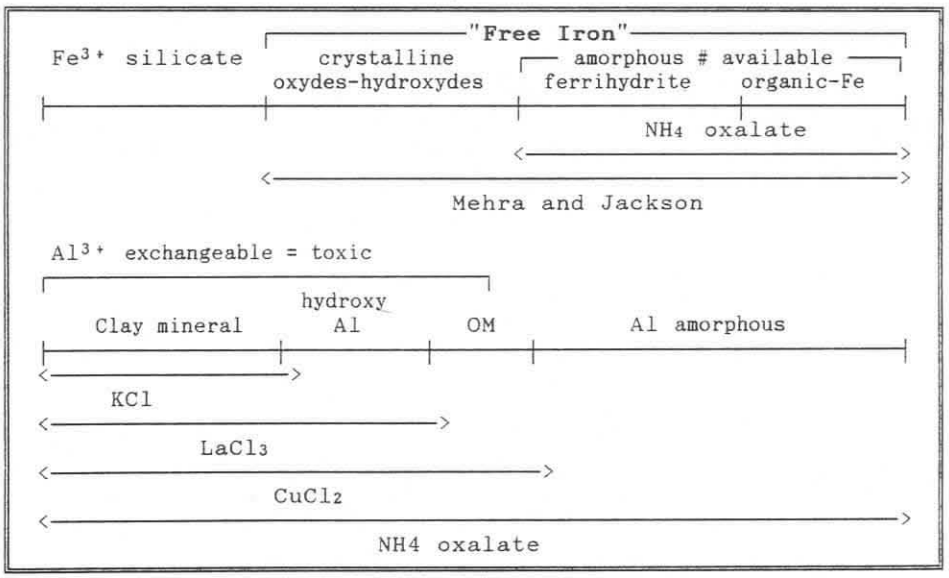
Iron Speciation in Soils. Large amounts of total and free iron are present in all kinds of soil, but in aerated soils, this iron is usually in the Fe^{3+} form. Iron is present in silicates, oxides and hydroxides, and in a third form that is of low-range crystallinity (amorphous form) and is, thus, more available. In the latter form, it is also possible to distinguish iron linked to inorganic or organic compounds. Although the content of amorphous iron is less than 1%, this is a significant amount. Iron speciation (Table 4) under these different forms can be deduced from various chemical treatments: treatment according to Mehra and Jackson [173] will give "free iron" (oxides + hydroxides + amorphous). Treatment with NH_4^+ oxalate in the dark yields the amorphous form [174,175].

Table 4 Chemical Data on Iron and Aluminum

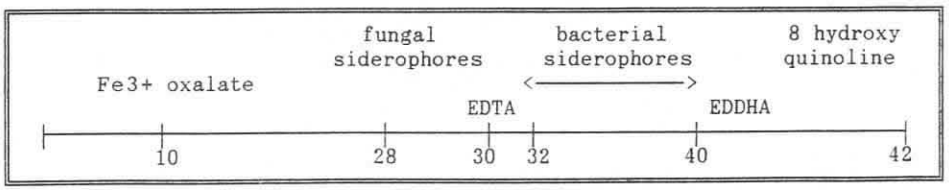
a) pk_a Values and Behavior of the Different Elements.



b) Phase Identification by Selective Dissolution Techniques.



c) Stability Constants (in log_{10}) of Various Natural and Synthetic Complexes of Iron.



The general behavior of iron in soils is dependent on its chemical characteristics, especially the pKa values of Fe^{3+} and Fe^{2+}, and the conditions of the environmental medium relative to oxidation—reduction and complexation [176]. In terms of the pKa values (see Table 4), Fe^{3+} is mobile only at very low pH values (pH < 3); thus, in the normal range of soil pH, the solubility is directly dependent on the very low solubility of the various oxides or hydroxides present, with solubility product (K_S) close to 10^{38}. Amorphous forms or ferrihydrite are relatively the most soluble compounds [176]. Reduction enables the formation of Fe^{2+} (pKa 9.5), which is mobile in the normal range of soil pH. Consequently, if microorganisms and plants are able to reduce Fe^{3+}, they can have an advantage in competition for available iron.

The other means by which iron can be solubilized is to form complexes. If these complexes are "chelates," they can mobilize iron over a wide range of pH. Microorganisms can secrete numerous low-molecular-weight organic acids in a relatively high concentration, the most frequent being oxalic acid from fungi. Even though the stability constant of oxalic acid is relatively low ($K_C < 10^{10}$), it can solubilize amorphous iron, or even other forms of iron, in relatively large quantities. Microorganisms also secrete very strong chelates (siderophores), but in very low concentrations.

Two environmental factors also have to be considered. The first is that even a very strong complex cannot alone release an insoluble element, and protons are generally required for the reaction. In the absence of protons, the complex can form only by precipitation of the organic ligand on the surface of the insoluble iron compound.

The second factor is for soils with relatively high pH values (> 7), in which $CaCO_3$ is present. This leads to the specific phenomenon called plant "chlorosis" [177]; even if all the causes are not completely elucidated, it is linked to iron bioavailability. Under these conditions, iron has the same low solubility as at lower pH (from 3 to 7) but the presence of $CaCO_3$ renders inoperable most of the acid or complexing secretions. Under these conditions, one of the best procedures for determining iron availability in plants is extraction with diethylenetriamine pentaacetic acid (DTPA) [178]. The iron content obtained is very often well correlated with the "amorphous" content.

Availability of Iron in Relation to Soil Suppressiveness to Diseases. Soils suppressive to some of the most important diseases caused by soil-borne plant pathogens have been described from different parts of the world [179]. Among the best known are the soils suppressive to fusarium wilts which can limit the severity of these diseases in banana, carnation, cucumber, cotton, flax, muskmelon, and tomato.

One of the first correlations established between suppressiveness and physicochemical factors concerns the presence of smectitic clays in soils suppressive to fusarium wilt of banana in Central America [155]. More recent examples involve the soils of the Salinas Valley in California and those of Chateaurenard in France, which are naturally suppressive for fusarium wilt. Very often these suppressive soils are compared with conducive soils in which the disease develops. Recently, other soils suppressive of tobacco black root rot by *P. fluorescens* were also described [157].

In the first example (Salinas Valley), the hypothesized mechanism is a competition between *Fusarium* and *Pseudomonas* for iron [180–182]. In the second example (Chateaurenard), the initial hypothesis was an intraspecific competition between the pathogenic and the nonpathogenic strains of *Fusarium*. It was also demonstrated that competition for carbon, related to the activity of the microbial biomass, was also involved in the mechanism of suppressiveness. More recently, however, the presence of montmorillonite [156,183] or vermiculite [157] and iron availability have been suggested [184]. It is worth noting that most suppressive soils have a pH value above 7 and contain a certain amount of $CaCO_3$.

Laboratory experiments showed that it was possible to inhibit the suppressiveness (i.e., enable the fungus to develop in the suppressive soil) if iron was furnished as a complex with a K_C (stability constant) less than 10^{30} (i.e., with ethylenediaminetetraacetic acid; EDTA). Introduction of a ligand, such as ethylenediaminedi[*o*]hydroxyphenylacetate (EDDHA), which has a K_C close to 10^{40}, enhanced the suppressiveness and converted a conducive soil to a suppressive soil. Fungi and bacteria can mobilize iron by the production of siderophores. Fungal siderophores have a K_C for iron close to 10^{28}, whereas different bacterial siderophores secreted by different species of the genus *Pseudomonas*, with catechol or hydroxamate groups, have a K_C from 10^{32} to more than 10^{40}; they have been called pseudobactin or pyoverdine [185,186].

The main forms of iron that occur in soils and the main K_C of the siderophores or artificial complexes that have been used in various experiments are presented in Table 4, and the structure of pseudobactin is shown in Figure 17 [185]. The ability to release iron is often measured by reference to a very strong complex of Fe (i.e., 8-hydroxy quinoline; $K_C > 10^{42}$).

This competition for complexing iron has been confirmed in a wide range of in vitro experiments, but questions arise related to the natural forms of iron in soils.

Although the iron competition system between bacteria and fungi can be reproduced in the laboratory, it is more complicated in the field as the result of the participation of other constituents such as $CaCO_3$, other kinds of acids, and even of several chemical reactions

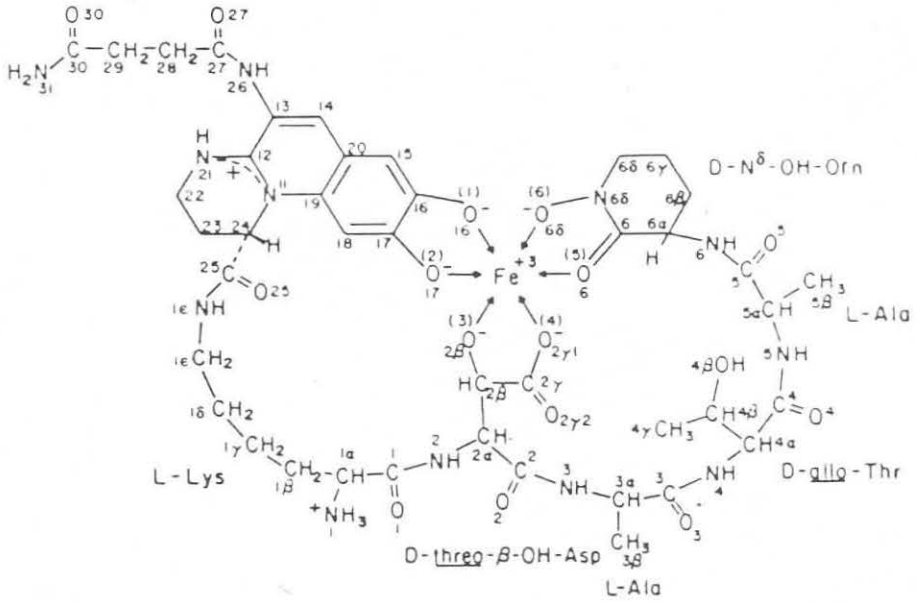


Figure 17 Structure of a siderophore: the pseudobactin. From Ref. 185.

(e.g., dissolution by H^+, iron reduction or complexation). Nevertheless, specific strains of *Pseudomonas* apparently suppress some diseases in soils caused by the subspecies *F. oxysporum* and by *Gaeumannomyces graminis* var. *tritici* [180]. Similarly, introduction into soils of plant growth-promoting rhizobacteria (PGPR), which belong to the genus *Pseudomonas* and are also able to produce siderophores, seems to increase the yield significantly [187]. Even though a better knowledge of siderophore behavior in the presence of $CaCO_3$ and other soil constituents, such as smectites [188] is necessary, such introduction of suppressive bacteria into soil represents a trend for the future.

Effect of Aluminum: A Major Stress for Microorganisms

According to Wright [189], one-fourth of the soils of the world are acid, and soil acidity is increasing for different reasons both in northern and southern parts of continents. So acidity represents a major chemical constraint for either plants or microbes, and all the data converge to focus on the specific role of aluminum in toxicity.

Soil pH and Aluminum Speciation. In the weathering process, a decrease in the pH of soils is normal. For example, in a 30-year field experiment in Versailles on silty soils, the pH decreased from 6.4 to 5. One of the main sources of acidity, although it is weak acidity, is CO_2 dissolution in water. Pure water has a pH close to 7, and the pH of water in equilibrium with the atmosphere at 25°C will not be lower than 5, even if the CO_2 concentration is high. The CO_2 concentration in the atmosphere, which is approximately 0.03%, has been higher in the past and might increase in the future; CO_2 is also derived from the respiration of living organisms and from the mineralization of organic matter. To obtain a pH lower than 5, stronger acids are necessary. These can be organic acids, formed during the degradation of organic matter (low-molecular-weight aliphatic acids are usually the strongest) or secreted and excreted by living organisms. The strongest acids, H_2SO_4 and HNO_3, that exist in soils, are inorganic, and they are formed through the oxidation of S or N compounds, which can be present in parent rock material (for S), in acid rain, in fertilizers, or in organic matter. The oxidation of NH_4^+ during nitrification is one of the main sources of acidity.

In soils, H^+ ions or protons will exchange for other cations such as Ca, Na, or K, present either in primary minerals (dissolution process) or on the exchange complex of clay minerals. When the pH is close to 5, the value of the pKa of Al, this cation can be released and will become the main internal source of acidity. It

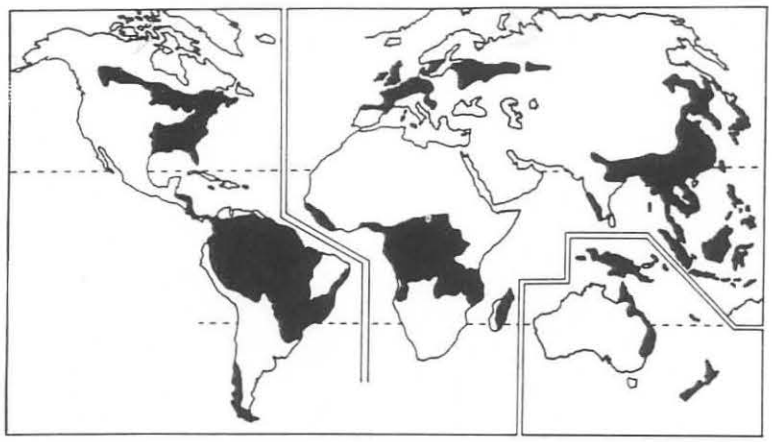


Figure 18 Major areas of naturally acidic soils. From Ref. 189.

first appears on the exchange complex, as it is derived from aluminosilicates, especially from the octahedral layer of phyllosilicates. The part occupied by Al^{3+} (and H^+) represents the amount of desaturation, which is a part of the cation-exchange capacity. Aluminum is also present in solution as several species (see Table 4), depending on the hydrolytic reactions. Soluble Al, even at low pH values, is more toxic than Al fixed on clays in an exchangeable form, but an equilibrium exists between the different forms of Al. Speciation of Al in solution can be measured either by nuclear magnetic resonance (NMR), which permits the differentiation of monomeric from polymeric species, or by spectrocolorimetric methods using aluminum or Ferron [190,191], and modeling is possible [192].

To determine the speciation of Al on a solid phase, different solutions can be used: (1) Al is displaced from exchangeable positions by KCl solution; this will give exchangeable monomeric Al (or $Al^{3+} + H^+$, if done by titration), the presence of which is well correlated with soil pH. This solution can also be used to determine other forms of acidity linked to the surface of soil constituents [173,193]. (2) Lanthanum or copper chlorides can exchange Al fixed on hydroxy-Al or organic matter. Stronger extractants, such as sodium citrate [194] are necessary, for the extraction of Al-polymerized interlayers, or ammonium oxalate for amorphous Al in allophanes and imogolite. Tetraborate is used for titration of Al linked to organic matter. Toxic Al corresponds to soluble and exchangeable Al^{3+}, but equilibrium exists with the other forms.

Aluminum Toxicity. Many experiments have shown that living organisms, especially microorganisms, can be grown in acid solutions without serious damage, suggesting that they are not too sensitive to the direct effect of H^+, but rather to the indirect effect of metal toxicities. Even if Mn is more available at low pH, Al toxicity is the most important limitation to plant development in many soils of the world, particularly in forest soils in the northern portions of the continents or in tropical and subtropical soils. The toxicity of Al to plants has been well documented for many years, but the effect of Al on microorganisms is less known, even though it is a major ecological constraint.

Toxicity of Aluminum to Fungi. Most studies on the toxicity of Al to fungi have dealt with either mycorrhizae or fungal plant pathogens, and the experiments sometimes involved both Al and Mn fungitoxicity [195]. In the case of mycorrhizae, studies have been concerned with the ability of fungi to develop in very acid medium (e.g., colonization of soils impacted with acid mine drainage or with acid rain). Thompson and Medve [196] classified the sensitivity to

Mn and Al of different ectomycorrhizal fungi (*Cenococcum graminiforme, Telephon terrestris, Pisolithus trinctorium*): Mn was less toxic than Al, and most of the fungi could grow in solution at Al concentrations between 250 and 500 ppm. According to these authors, such mycorrhizae might be used to confer Al tolerance in the reforestation of acid mine soils or areas affected by acid precipitation. Firestone et al. [195] analyzed fungitoxicity in relation to acid rain and confirmed the nontoxicity of Mn. Spore germination was found to be inhibited by relatively high concentrations of Al (>500 ppm), the toxicity being reduced by the addition of a complexant, such as fluorine, to the Al solution.

Ko and Hora [197] showed that fungistasis is related to Al toxicity in the Hawaiian Islands, where it is widespread. Also in Hawaii, Kobayashi and Ko [198] and Ko and Nishijima [199] showed that soil suppressiveness towards *Phytophthora capsici* and *Rhizoctonia solani* seemed to be related to soil pH. They analyzed both the nature of the suppression and the mechanism of lysis of fungal mycelia in soils. Orellana et al. [200] showed that *Verticillium alloatrum* was almost completely suppressed by 8 ppm in vitro: hyaline unpigmented mycelia and very few microsclerotia were present. At pH 4.7 or below, *Whetzelina sclerotiorum* was more tolerant to Al.

Recently, a study was performed in France on the reactivity of soils to *Fusarium solani* or *F. coeruleum*, the principal causes of dry rot in France and South America. Suppressive soils are relatively abundant and all have a low pH (<5.5) [201]. Soil suppressiveness, which is linked to the presence of a higher content of monomeric Al than in conducive soils, can be removed by raising the pH to 5 or above by liming [202]. It was demonstrated that it was also possible to confer suppressiveness by soil solutions or Al^{3+} solutions, but not in the presence of fluorine which complexes with Al. With use of a microprobe, Al was identified on the macroconidia that developed in the suppressive soil extract [202]. Research conducted in Amazonia [203] demonstrated that naturally acid forest soils are suppressive for different species of *Pythium*, but the soils became conducive under cultivation.

Hence, some species of fungi are very resistant to aluminum and are able to resist up to a 100 ppm of Al, whereas others are very sensitive, and 1–10 ppm in solution is enough to lyse the cells. It seems that aluminum can be a major cause of fungitoxicity and of disease suppressiveness.

Action of Aluminum on Bacteria. Zwarum et al. [204,205] measured the cation-exchange capacity of bacteria and found values from 95 mEq 100 g^{-1} of cells (oven-dried weight) for *Bacillus* to 340 mEq 100 g^{-1} for *Pseudomonas stutzeri*, which is smaller in size. The

first organism was inhibited by increasing acidity, but no further detrimental effect was produced by the addition of up to 80 ppm of soluble Al. However, upon saturation with Al, there was a change from gram-positive to gram-negative, and *P. stutzeri* (gram-negative) was more sensitive to Al (10 ppm).

The response of rhizobia to both pH and Al has also been studied. The effects also seem to be complex and differ among strains: slow-growing rhizobia tend to be less acid-sensitive than fast-growing ones, although there are some variations and exceptions [206]. The action of Al reduced the frequency of cell division [207,208]. Acid-tolerant strains of rhizobia seem to produce more exopolysaccharides than do acid-sensitive strains [209]. However, Cunningham and Munns [210] found no correlation between total buffering or chelate ability of Al and production of EPS. Hence, an explanation has to be found, which may be at the level of the charge of the EPS, as such knowledge would be useful for the introduction of selected strains of bacteria, especially of rhizobia, into soils [211].

The toxicity of different species of Al is not yet clear [208]. Work has to be pursued with better control of mono- versus polymeric species, otherwise the exact mechanisms of Al toxicity to bacteria cannot be understood. Another important aspect of bacterial ecology in acid environments that requires clarification is the role of acidity and Al in nitrification. The first range of pH was defined for pure culture of nitrifying bacteria and limits are shown: there is an increase of the rate in nitrification with increases in pH from 4 to 8 [212]. Acidification tends to depress, but not to eliminate, nitrification, which can be because there is less microbial activity at low pH.

There is a general effect of acid rain on the decrease of microbial decomposition rate of organic matter and transformation of N in acid forest soils (ammonification, nitrification, and denitrification) [213, 214]. However, contradictory data exist: several authors [e.g., 215] have recently pointed out that, in forest areas where acid rain is high, nitrification can be very effective even at pH less than 4, and a correlation can be established between the high amount of nitrate in solution and the release and migration of Al. Thus, this nitrification process, which occurs seasonally, is the cause of supplementary soil acidification [216].

In soils, the phenomena are complex, and Boudot et al. [217] demonstrated that Al linked to organic matter can decrease OM mineralization or nitrification, either by chemical (chelate formation) or physical protection of OM. Such phenomena are important for many soils in which OM accumulates, and intervention of Al toxicity can also be suspected.

As with water potential, pH and Al levels are important environmental factors that can effect the antagonism of fungi by bacteria

in soils [218,219]. It should be noted that, as with plants, monomeric Al^{3+} seems to be responsible for the toxicity [220]. For plants, various mechanisms of the toxicity of Al are hypothesized, and they can act differently in different species.

1. Coprecipitation with, or adsorption onto phosphate
2. Inhibition of enzymes and growth regulators [221]
3. Fixation on DNA or RNA and blocking their replication [222]

In microorganisms, even if the exact mechanisms of toxicity are not known, the fixation of Al seems to be external, which emphasizes the role of exopolysaccharides or of exchange capacity of the organisms on the suppression of toxicity.

Natural chemical stresses, such as acidity and salinity, cannot be prevented, and solutions need to be found, since these problems pose major constraints on agriculture, particularly in developing countries. For acidity, liming is certainly the main remedy, and it will have the greatest effect on microbial life. Where liming is possible, it will be a remedy both for Al toxicity and some heavy-metal pollution. However, we have already seen that liming can lead to some new diseases and problems with iron bioavailability. For both Al and salinity genetically adapted microbes and plants would certainly be the best solution.

IV. INFLUENCE OF MICROORGANISMS ON THEIR MINERAL ENVIRONMENT

A. Role of Microorganisms in Weathering of Soil Minerals

The overall roles of biological and biochemical factors in the weathering of soil minerals has been recently reviewed [223]. In this chapter, some complementary data on microbial aspects of weathering will be emphasized.

The general role of microorganisms in weathering, which occurs through biochemical actions, must be distinguished. Microorganisms are the main cause of oxidation–reduction reactions, which, for the microbes, are a source of energy. The most general phenomenon is OM mineralization, which results in organic acids, CO_2, or NH_4^+. These compounds will modify the general weathering conditions by providing an acid pH that can range from 6 to 5 with CO_2, to less than 5 with organic acids, as well as the formation of complexes and chelates. Microbes are also the main oxidizers of NH_4^+, S, and FeS_2, which results in the production of HNO_3 and H_2SO_4. The presence of such strong mineral acids can explain local pH values of less than 3.

Thus, the weathering of rocks and minerals is increased, making the mobility of metals, such as Al^{3+}, Fe^{3+}, or other transition metals, possible.

In such a general weathering system, which represents a "macrosystem," living microorganisms are one of the four principal factors in soil formation, besides parent rock material, climate, and time [224]. Important soil processes, such as acidification and podzolization, which are located in cold regions of the world (e.g., northern part of continents and mountain areas), are also associated with such biochemical factors related to microorganisms [225,226].

Another more direct role of microorganisms in a weathering microsystem will be illustrated; as in aggregation, the influence of microbes is somewhat limited to the immediate and surrounding environment. Some experimental results illustrate how this microsystem works [227]. In the first experiment, several fungi (*Curvularia lunate, Sclerotina sclerotium, Sclerotium minor,* and *Aspergillus niger*) were grown on agar that contained glucose, yeast extract, and Ca-saturated vermiculite. After a few days, SEM observations showed both dissolution patterns and abundant precipitates located on the vermiculite or around the fungi. These precipitates, as determined by microprobe and X-ray diffraction, were composed of calcium oxalate (Figure 19). Thus, fungi are able to secrete abundant quantities of oxalic acid and causes Ca to be released. In the second experiment, the same fungi were grown in the presence of crystalline iron phosphates (vivianite). After a few days, observations with SEM showed that dissolution of the vivianite had occurred, and precipitates were again located around the hyphae of the fungi (see Figure 19). Electron microprobe analysis identified the precipitate as a secondary iron phosphate.

These experiments demonstrated that the general microbial microsystem consists of solubilization–precipitation processes that occur on a small scale. Figure 20 shows schematically that these processes include various mechanisms [223]. One of these mechanisms involved in solubilization is cation exchange (for example the Ca of the vermiculite) with the different charged sites of the external part of the microbe [228]. Most often, H^+ is involved, so the exchange reaction is difficult to isolate from acid dissolution mechanisms. Low-molecular-weight organic acids are also excreted by microbes: oxalic acid is frequently excreted by fungi and lichens, but citric and 2-ketogluconic acids are also reported. Under certain conditions, the production of oxalic acid by fungi and mycorrhizae and its precipitation as calcium oxalate can be so abundant that it constitutes a calcium "reservoir" for "calcicol" flora in subsurface horizon developed from acidic rocks (Callot, unpublished data).

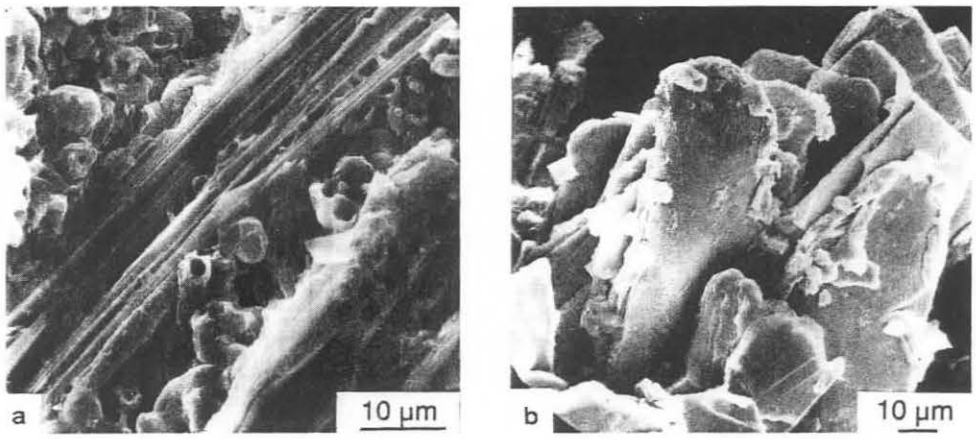
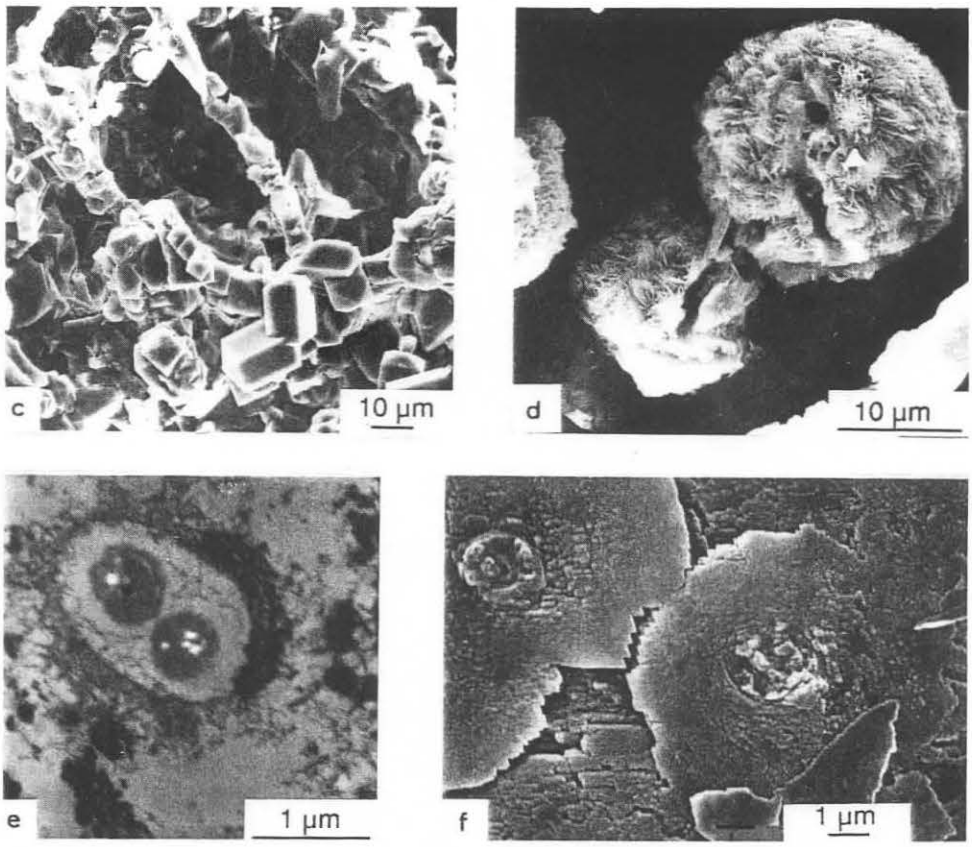


Figure 19 Microbial weathering of soil minerals: (a) Lichen hyphae invading a crystal mica; (b) precipitation of Ca-oxalate on Ca-vermiculite; (c) precipitation of Ca-oxalate around the hyphae; (d) precipitation of secondary iron phosphate around the hyphae; (e) precipitation of iron on exopolysaccharide around a *Lepothrix* bacteria; (f) precipitation of $CaCO_3$ around a bacteria (the bacterium is at the center). Low-temperature scanning electron microscopy. From (a) [223]; (b, c, d) J. Thompson et al., unpublished; (e) [227]; and (f) [229].

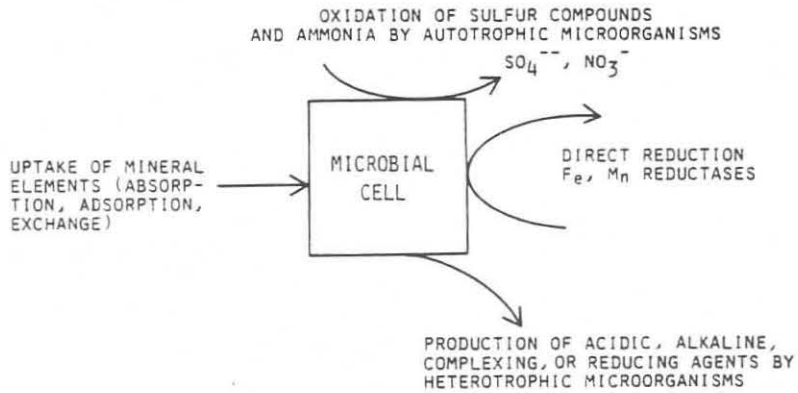
Although microbes can have an important role in dissolution–precipitation of phosphates minerals, such phenomena are also important for carbonates (see Figure 19) [229]. In certain cases, specific bacteria (e.g., *Alcaligenes eutrophus*) can even precipitate heavy metals around them under a carbonate form [230]. In the case of silicates, an increase in solubility generally occurs with acid and complex secretions, and feldspars or micas can be destroyed (see Figure 19). However, the existence of specific microbes has not been proved (e.g., the involvement of *Bacillus siliceus*, quoted by several authors, has not been definitively demonstrated). Specialized microbes do appear to be involved in the transformations of sulfur, iron, and manganese. Sulfur bacteria (*Thiobacillus*) have a major role in mangroves and sediments, and iron and manganese bacteria are of major importance in the dynamics of these elements in soils and sediments. Berthelin [226] has shown the role of bacteria in iron reduction and that an enzymatic mechanism similar to dissimulative nitrate reduction is



involved. Lefebvre and Rousseau (1991, unpublished data) have shown that *B. polymyxa* grown at pH 6 is far more effective in dissolving goethite and hematite than are concentrated mineral acids (N/10 or N), even with the addition of a reductive agent. Oxidation of Mn and Fe and their precipitation inside or around bacteria such as *Gallionella* or *Leptothrix* are frequent phenomena [231].

In several of these examples of the precipitation of elements around cells, exopolysaccharides seem to have a major role in the initial steps of readsorption of the element (see Figure 19). Although siderophores are of great importance in iron bioavailability, they are probably of minor importance, in terms of weathering, because of their low concentration.

In summary, many microbes are able to weather minerals that are located in their ambient environment, mostly by the secretion of



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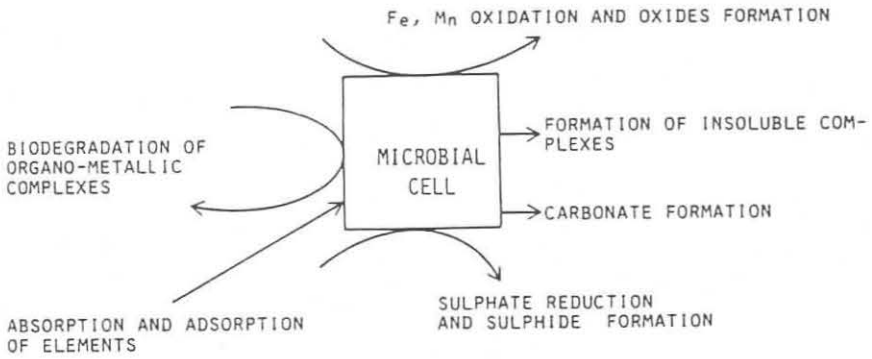


b

Figure 20 Soil microbial microsystems in weathering: (a) dissolution; (b) precipitation. From Ref. 223.

low-molecular-mass organic acids. In precipitation of the element, polysaccharides are often involved. Very often microbes will derive nutrients, and sometimes energy, from the reactions involved. Such factors should be considered relative to the effects (see Section IV.B) of the majority of microbes on soil organization, which is mainly microaggregation through polysaccharide production.

B. Microbially Mediated Aggregation in Soils

That microorganisms have a major role in soil aggregation was recognized in the early 1940s by Waksman and co-workers, and studies

on this topic have been regularly reviewed [e.g., 232–235]. Evidence for aggregation by microbes came from studies on the incorporation of readily utilizable organic matter in soil [e.g., 231–235] and from studies in which selected microbes were grown in soils or minerals and their effects on aggregation investigated [238,241–244]. Both types of studies showed significant increases in water-stable aggregates. Fungi, rather than bacteria, appear to have the predominant role [232,240,244–246], but efficiency is variable among species [e.g., 237]. Microorganisms contribute to the reorganization and coalescence of mineral particles into new aggregates (i.e., to the molding of aggregates) [247]. They are also involved in the water-stabilization of existing aggregates. In turn, good soil structure enhances biological activity. The role of roots and fauna in soil aggregation will not be considered here, although this separation is quite artificial, as the main concentrations of microbes in soils occur in relation with plant remnants, the rhizosphere, and faunal excretions. The aggregating action of the rhizosphere, earthworms, and termites owes much to microorganisms [47,248–252].

General Mechanisms Involved in Aggregation by Microbes

Several mechanisms have been hypothesized to explain how microorganisms can enhance the formation and stability of soil aggregates. These include the mechanical binding of soil particles by filamentous microorganisms, the adhesion of microbes to soil particles, and the production of aggregating substances (e.g., polysaccharides).

Reorientation of Soil Particles and Mechanical Binding. Changes in the soil or the clay fabric can be observed in the vicinity of fungi or bacteria. When fungi were cultured in clay pastes (at a constant water potential), the clay platelets were observed to be reoriented parallel to the microbial surface and compacted (Figure 21a) [253]. These effects presumably result from the pressures exerted by the fungi during growth, and from local clay shrinkage associated with the adsorption of water by the microbes. Extensive polysaccharide secretion occurred in these experiments (see Figure 21b), and the microorganisms became surrounded by a microenvironment that they constructed [47]. After desiccation and rehydration, cracks originate at the boundary of this microenvironment and tend to separate it from the soil mass (see Figure 21c,d), which represents the first steps in aggregate formation and corresponds to Allison's [247] molding of microaggregates. In the case of bacteria or yeasts, preferential orientation of clay minerals parallel to the cell wall was also observed in soil aggregates [123] or in clay matrices [253]. It is

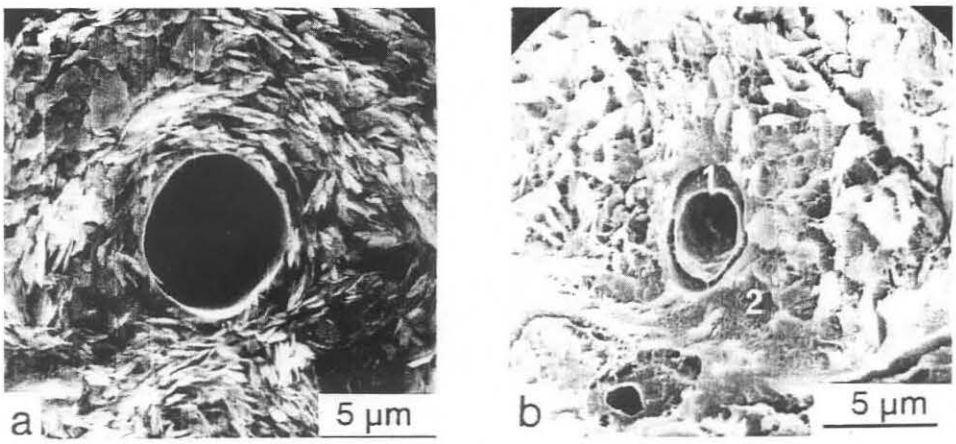
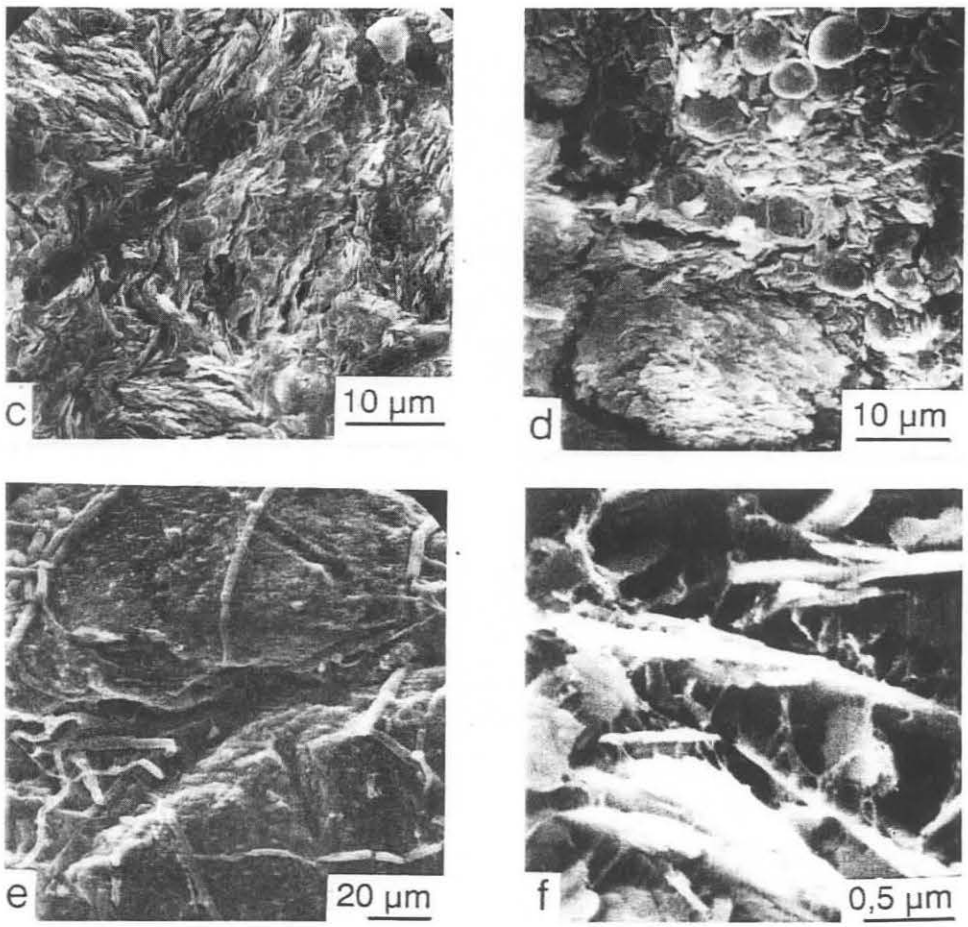


Figure 21 Microbially-mediated fabrics in clay minerals. (a) Parallel orientation of kaolinite platelets around hyphae of *Chaetomium* (−0.01 MPa); (b) polysaccharide secretion in fungal culture in kaolinite (*Chaetomium* sp.) (−0.01 MPa); (c) fissures in rehydrated kaolinite colonized by *Trichoderma* sp. (−0.01 → −100 → −0.01 MPa); (d) fissures around a yeast colony in kaolinite (*Saccharomyces* sp.) (−0.01 → −100 → −0.01 MPa); (e) physical entanglement of clay microaggregate by hyphae of *Chaetomium* (−0.01 → −100 → −0.01 MPa); (f) polysaccharide strands in kaolinite–fungal polysaccharide (scleroglucan) complex (−0.0032 MPa); low-temperature scanning electron microscopy. From Ref. 253 (a–e); Chenu, unpublished (f).

probable that the orientation and compaction of clay minerals in the vicinity of microorganisms results mainly from the shrinkage of the clay during desiccation in soil.

At another level, fungi growing on natural or on artificial aggregates made of clay or sand–clay mixtures [237,248] can be observed to form a network of hyphae all over the aggregate (see Figure 21e) [236,242,245,248,253,254], and Tisdall and Oades [248] found a good correlation between the length of the hyphae of vesicular arbuscular fungi and the water stability of aggregates. The increased stability of aggregates inoculated with fungi was, thus, ascribed to the physical retention of the soil particles by the hyphal network; that is, it was assumed that a network of fungal filaments is sufficient to protect the aggregates from slaking upon sudden wetting or from the dispersion of soil particulates. In addition, hyphae are frequently



covered with a mucilaginous layer, presumably of polysaccharides [250], as many fungi are known to produce extracellular polysaccharides to which the clay platelets adhere [243,248,250]. To evaluate both actions, Aspiras et al. grew fungi on soil aggregates and later disrupted the mycelial network by sonication [241]. Most aggregates remained water-stable, demonstrating that binding factors other than the mechanical effect alone were involved, among them some mucilaginous secretion by the fungi. Similar mechanisms presumably occur with other filamentous microorganisms, such as actinomycetes.

Adhesion. The adhesion of microorganisms to mineral particles has often been argued as a possible mechanism in aggregation by microbes [230]. As indicated by Lynch and Bragg [235], three cases happen in soils: adsorbent mineral particles can be larger than microorganisms (e.g., silts and sands), of equal size, or even smaller (e.g., clay minerals, micromicas, very fine quartz particles). In the latter, it is assumed that if several mineral particles adhere to bacteria, the overall association is a microaggregate (i.e., the microbial cell acts as a binding agent) [235]. Actually, clay minerals coating bacteria are a quite common feature in thin sections of soil samples [121–123,257]. Are such associations the result of adhesion processes?

As discussed by Stotzky [1], there is abundant empirical evidence for surface interactions between soil microorganisms and solid surfaces, as microbes are not leached out of soil horizons, but are retained. However, there are few laboratory experiments that demonstrate the ability of bacteria to adhere to clay minerals [66,258, 259]. The mechanisms involved in the adhesion of microorganisms to solid surfaces are physical and nonspecific [1,82,260,261]. They include van der Waals interactions, electrostatic interactions (DLVO theory applied to adhesion) [262], hydrogen-bonding, as well as hydrophobic interactions (thermodynamic approach) [263]. It is often assumed that at the ambient pH of most soils, clays and microorganisms are predominantly negatively charged [82,260]. Therefore, microorganisms must overcome an energy barrier before intimate contact can be achieved, and the electrostatic repulsive forces must be counterbalanced by van der Waals attractive forces. Several mechanisms have been assumed to render this adhesion possible; these are the occurrence of low pH (lower than the pKa of microbial cells) in the vicinity of the cells [260]; the presence of polyvalent cations [82]; and the secretion of polysaccharides that act as binders [264,265]. Surface hydrophobicity (of the microbe or of the solid substratum) may also help adhesion, but this has been given less attention. As demonstrated by Moses and co-workers [266], the adhesion of hydrophilic cells depends very much on electrostatic interactions, but less so for microorganisms with intermediate degrees of hydrophobicity, whereas a hydrophobic microbe, which was also strongly electronegative, adhered to negatively charged surfaces. Physical forces, developed in the drying of soils or in the growth of roots, might also bring the microbes and the clay surfaces close enough to overcome the energy barrier [1], or at least close enough for the exuded polymers to adsorb on the mineral.

Production of Aggregating or Water-Stabilizing Substances. The production of microbial substances is assumed to be a major mechanism

by which bacteria contribute to aggregation processes [232,234]. Abundant organic matter, mainly polysaccharides, is produced by microorganisms, as they have an intense secretory activity [117]. Moreover, the microbes themselves have rapid turnovers and hence, the dead cells are then subject to biodegradation, and their constituents are more-or-less quickly released in the soil. Although bacterial and fungal walls have been demonstrated to have high stabilities toward degradation [267–269], other cell constituents are rapidly degraded [269,270], and microbial cells were reported to have half-lives of several weeks [270,271].

A prerequisite for the involvement of organic compounds in aggregation is considered to be their direct interaction with soil minerals, namely, their adsorption on clay minerals. A wide range of microbial substances adsorb on clay minerals, depending on their molecular characteristics as well as on the charge and surface area characteristics of the clay and on the ambient environment. Results and theories on the adsorption of organic polymers on clay minerals have been analyzed by Theng [31,272] and are not discussed here.

Polysaccharides: Binding Agents. Polysaccharides are one of the most efficient fractions of soil organic matter in aggregation processes. A recent review of studies that have demonstrated their importance was provided by Lynch and Bragg [235]. The general trends that emerge from the abundant literature on the topic are the following: Microbial and root-derived polysaccharides are considered to be more active in aggregation than other plant polysaccharides. It is likely that polysaccharide constituents of fungal walls, such as chitin or glucans, are not aggregating agents, as they are essentially nonsoluble and rigid molecules. However, partial biodegradation can change these characteristics. Extracellular polysaccharides (i.e., capsular or slime layer polysaccharides) seem to be the microbial compounds most active in aggregation.

Studies on the mechanisms by which polysaccharides contribute to soil aggregation have been performed with model polysaccharide–mineral (clay) associations. They will be discussed in the following section.

Since polysaccharides are linear macromolecules with long and flexible chains, they were generally hypothesized to adsorb on several mineral particles at the same time and, therefore, bind them [272–274]. This polymer-bridging concept is based on studies of adsorption–flocculation reactions of clays–synthetic polymer in suspension [272,275,276], and on other considerations:

1. Many polysaccharides adsorb on clay minerals, and a general feature is that anionic polysaccharides, which are predominant among EPS, adsorb on clay minerals only at low pH

(<4) or in the presence of di- or trivalent cations. Neutral polysaccharides can be readily adsorbed in high amounts and with high affinity [277–286].

2. Adsorbed polysaccharides can flocculate clay minerals [284, 287,288].
3. Low temperature SEM of clay–polysaccharide complexes allows the polysaccharide strands to be visualized (see Figure 21f) [124,285].
4. Adsorbed microbial polysaccharides reduce the dispersability of clay [285,289].
5. They increase the interparticle cohesion of clay minerals [290]. The tensile strength of a kaolinite increased with the amount of adsorbed polysaccharide. The maximal strength (20 times that of pure kaolinite) was attained when approximately 60% of the surface area was covered by the polysaccharide. Above this threshold, every clay-to-clay contact was presumed to occur through polysaccharide bridges.

The effectiveness of polysaccharides in the bridging process was related to their molecular weight and conformation [272,273,291]. Research on properties and applications of polysaccharides have highlighted other major characteristics of these polymers: In many polysaccharides, intermolecular physical linkages occur (hydrogen bonding, van der Waals forces) that lead to macroscopically viscous solutions and gels [292,293]. It has been observed that polysaccharides can form gels, even when adsorbed on clay minerals [284]. Hence, the cohesion of clay–polysaccharide associations results mainly from an organic network in which the clay particles are embedded (i.e., organomineral gels occur; Figure 22).

The adsorption mechanisms of polysaccharides on clay minerals can be predominantly related to their primary level of structure, but the bridging process depends mainly on their tertiary (shape) and quaternary (intermolecular linkages) structures. Polysaccharides with ordered helical conformations (e.g., xanthan, bacterial alginate, β-1,3-glucans, galactomannans), which form viscous solutions or even gels and have high water-retention properties [292–294], seem to be the most effective in bridging. These polysaccharides are abundant among the extracellular polysaccharides of microbes, as they are major constituents of many capsules and slimes [295–297].

Because polysaccharides that have intermolecular linkages have the highest viscosities, the foregoing hypothesis of organomineral gels might contribute to explaining why the viscosity of the

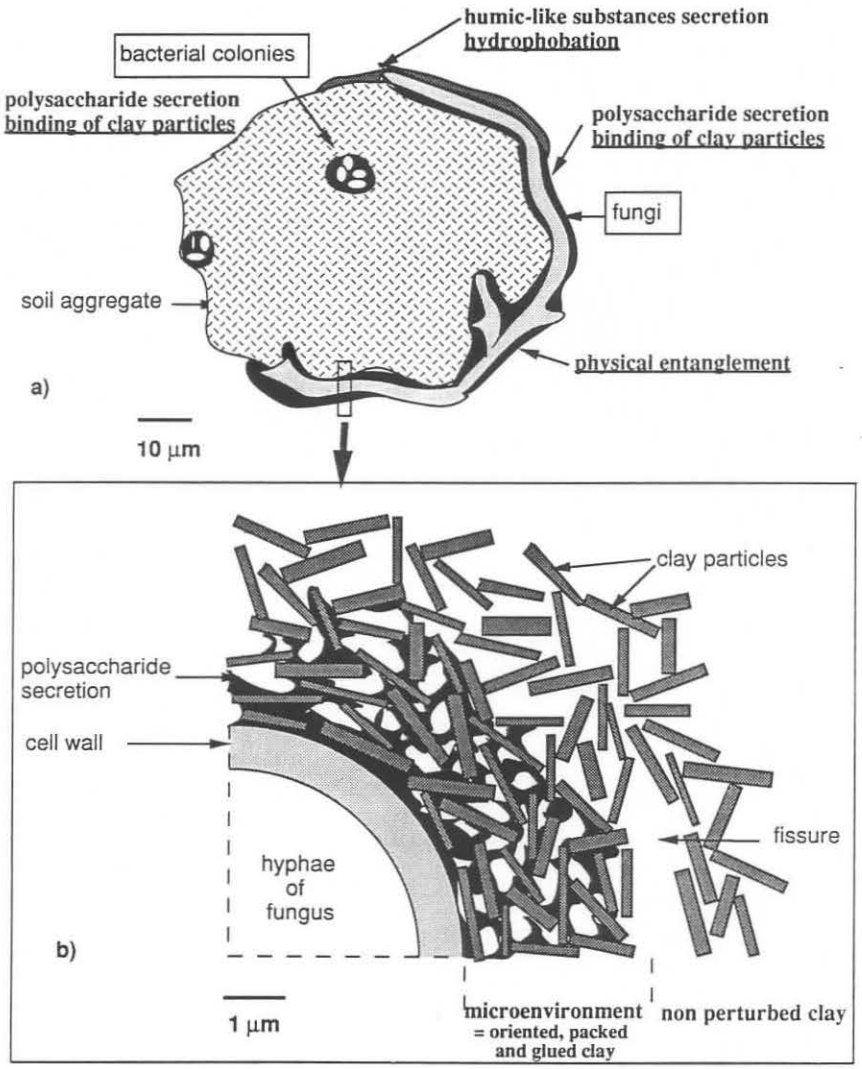


Figure 22 Microbially mediated aggregation: (a) Schematic representation of the binding and stabilization of a soil aggregate by microorganisms; (b) detail of the microenvironment in the vicinity of a fungi. (From C. Chenu and J. H. Dorioz, unpublished.)

polysaccharides in solution was a molecular characteristic that correlated fairly well with the effectiveness of these polysaccharides in aggregation [298,332]. In the experiments of Channey and Swift [299], bacterial alginate probably formed gels between the soil microaggregates and then established "bridges" on a larger scale to form bigger aggregates. The effectiveness of Ca in microbially mediated aggregation [300] could also be related to the ability of Ca to gelify many anionic polysaccharides by cross-linking [292].

Several points need further discussion to demonstrate how all this works in soil. First, do microbial strains that are known to produce polysaccharides in culture really produce them in the soils, where starvation conditions tend to prevail [1]? Electron microscopy of soils reveal that extracellular polysaccharides are widely present around bacteria and fungi [48,123,301], and many analyses of soil polysaccharide are consistent with the polysaccharides of microbial slimes or capsules [302].

Second, most experiments concerning the adsorption of polysaccharides on clay minerals and the flocculation and dispersability of the clay–polysaccharide complexes were conducted in dilute suspensions, in which the clay surfaces are accessible and the polysaccharide is in solution. Consequently, careful attention is necessary when extrapolating these data to soils, in which the mineral surfaces are not fully accessible to high-molecular-mass polymers [303,304].

A third point to be discussed is whether the polysaccharides are in solution when they interact with clays. It is more probable that extracellular polysaccharides are exuded by soil microorganisms in the form of gels or viscous slimes. From the observations of Foster [122,301], it can be deduced that extracellular polysaccharides of bacteria remain as discrete zones several microns thick around microbial bodies. Similar features were recorded with yeasts and fungi growing in clays [47] (see Figure 21b). As discussed by Oades [305], it is questionable whether extracellular polysaccharides occur as mobile polymers in soil, because of their physical state and affinity for clay minerals. Furthermore, the diffusion of polymers in soil seems to be very limited [303,304]. The concentrations of extracellular polysaccharides are probably very high in the vicinity of soil microorganisms, and it is possible that clay–polysaccharide interactions result from the adsorption of clays on polysaccharide gels and the embedment of clays in polysaccharide gels, as well as from the adsorption of polysaccharides on clay minerals.

Polysaccharides thus act as binding agents that enhance the resistance of aggregates to both slaking and clay dispersion. They are thus involved in aggregate formation as well as in aggregate stabilization.

Lipids and Humic-like Substances: Water-Stabilizing Substances. Another important class of microbial organic matter includes the lipids and humic-like substances. Their possible involvement in aggregation has received considerably less attention than have polysaccharides. Microbial lipids mainly consist of aliphatic hydrocarbons, ketones, wax esters, and very complex high-molecular-weight waxes [306]. Although bacteria, fungi, and algae generally contain less than 10% lipids [306], microbial lipids contribute to the stability of soil aggregates [307,308; G. Coulibali, doctoral, thesis Université de Poitiers, 1984] and are strongly associated with soil clay minerals [309]. Among the humic-like substances, the fungal melanins are of particular importance. These are intra- or extracellular dark-colored polymers that resemble humic acids in many characteristics (e.g., in elemental composition, functional group content, exchange acidity) [310–314]. Several authors have established, by using polar or nonpolar solvents, that the water-stabilizing action of various fungi is caused by such compounds [241,315,316].

Bond [245] and Monnier [236] demonstrated that when fungi were grown on soil aggregates, these aggregates became hydrophobic and water-stable. Microbial lipids and humic-like substances were presumably responsible for this. When lipids [317] or humic substances [318,319] are adsorbed onto clay minerals, water contact angles (θ) of more than 90° are recorded, whereas clean silicate surfaces are highly hydrophilic ($\theta \approx 0°$). The clay surfaces become hydrophobic at low organic contents (≈1%); i.e., at low coverage of the clay surface area [319]. Hence, humic-like substances can be expected to be quite effective in stabilizing the soil aggregates. Another important point is that the hydrophobic properties of organic compounds are expressed upon desiccation [315]. This has been interpreted as conformational rearrangements during desiccation, leading to the exposure of hydrophobic moieties on the molecules [320]. There are several other kinds of organic compounds (e.g., proteins) [Jouany, unpublished results] and polysaccharides [Chenu and Jouany, in preparation] that can induce some water repellency upon desiccation. Water repellency of soil aggregates reduces the wetting rate and, thus, the slaking of aggregates [236]; lipids and humic-like substances are, therefore, involved in the stabilization of existing aggregates.

To conclude, soil microorganisms can contribute to the formation and stabilization of soil aggregates by several mechanisms. The specific effectiveness of fungi in aggregation processes is the result of several mechanisms that are summarized in Figure 22: physical entanglement is associated with the secretion of binding substances (many fungi produce β-1,3-glucans that are very effective binders). In addition, many fungi also produce humic-like substances that can render the aggregates hydrophobic. Physical

entanglement and polysaccharide secretion are also involved with actinomycetes [242]. Bacteria act mainly through the secretion of aggregating substances, such as polysaccharides, or to a lesser extent, water-stabilizing ones, such as hydrophobic compounds. The role of bacterial adhesion in soil aggregation is yet to be confirmed.

In many cases, when aggregates stabilized by organic matter are allowed to dry or to settle for some time, an increase in water stability is recorded. This "ageing" phenomenon has recently been emphasized [250,321–323] and L. Habbib, doctoral thesis, Université de Nancy]. Thixotropy has been proposed as a mechanism [317]. Other factors that have been less considered in the literature might also be involved and may lead to increases in water stability (e.g., enhanced repellency of organics after desiccation and increases in organic versus mineral bonds as the constituents come closer together on desiccation).

Levels of Microbial Aggregation

Bacterial Microaggregates. In thin sections of soil, bacteria or bacterial colonies are frequently surrounded by polysaccharides [48, 301] and by oriented clay particles [121–123,263,324]. These features, described as microaggregates [305], were discussed previously in this chapter (Section III.A). Indirect evidence of such microaggregates has also been given: When small-sized fractions are separated from soils by physical dispersion methods, ultrasonic-resistant microaggregates remain in the <20-μm fractions [6,7,59]. Labile organic matter that is primarily aliphatic is characteristic of this-sized fractions [6,325] and coexists with highly condensed aromatic moieties [59,325], which suggests that both microbial substances and humic-like components are involved in the stability of these microaggregates. The TEM studies have shown that ultrasonic-resistant microaggregates mainly consist of bacterial microaggregates and, in the coarse clay fraction of a loessic cultivated soil, 90% of the enumerated bacteria were coated with clay minerals [Chenu and Balesdent, unpublished results]. Jocteur Monrozier et al. [59] demonstrated that 40–60% of the microbial biomass may be associated with the microaggregates of 2–20 μm in size, depending on the abundance and nature of the clay minerals. Even if bacteria make up only a few percent of the total organic carbon in most soils (0.1–5%), such microaggregates have a great significance because: (1) they persist after the death of microbes; (2) they control microbial efficiency of substrate oxidation [326]; (3) they limit the numbers of microbes enumerated [Richaume, personal communication]; and (4) successive microbial populations contribute to build up a stable pool of microaggregates rich in organic materials.

Fungi and Actinomycetes. Fungi are preferentially located outside of soil aggregates, as demonstrated by direct observation [236,245] or by washing–sonications of aggregates [2]. Hence, fungi stabilize larger aggregates than do bacteria (e.g., aggregates of several tens of microns in diameter) [47,58,327].

The size and shape of microorganisms and their secretion, if any, of aggregating substances determines the scale at which microorganisms act. Fungi and bacteria, through their secretion of binding substances, can generate aggregates in their microenvironment from a few microns for bacteria to several tens of microns for fungi [253]. Microbially mediated aggregation results overall in large stable microaggregates (>200 μm) [60,239,300,328, 329], which are composed of several hierarchical levels of particle aggregation, each level being the result of a major binding agent [58].

Persistence of Microbial Aggregation

Microbial aggregation is relatively transitory and decreases over several weeks [236,240,242,330,331], primarily as the result of the short life of the microbes themselves and because of the lability of their organic constituents, especially of polysaccharides [234,243, 270,332–334]. This lability has frequently been invoked to lessen the importance of microbial aggregation. It is, indeed, a serious handicap in the use of polysaccharides as soil conditioners. However, under natural conditions, microbial generations succeed each other and maintain an average level of aggregation, with seasonal variations [281].

Several factors related to soil minerals are likely to enhance the persistence of microbial compounds (e.g., metal complexation of polysaccharides [234,334] and adsorption of humic substances [332]), which are considered to be chemical mechanisms of protection. Physical mechanisms of protection (*physical* means that the protection can be suppressed by physical dispersion agents) have also been described: adsorption of the labile compounds on clay minerals [335–337] or on amorphous Al–Fe compounds [217] and occlusion within the clay porosity [303]. For example, the clay-coated bacteria or amorphous organic matter that are observed in ultrathin sections of soils are presumably protected from microbial degradation (see Figure 15) [48,122,333]; and many studies concerning organic matter turnover emphasize the physical protection of organic matter by entrapment in pores that are inaccessible to microbes [339–341]. A close relationship between the turnover of organic matter and stable aggregation can be postulated, as once aggregates are disrupted, the enclosed organic matter is available for degradation.

Consequences for Microbial Development

By the secretion of organic polymers and by mechanical and physical actions, microorganisms change the organization and physical characteristics of the media in which they live. However, little information is available on the influence of microbes on physical properties of soil, other than aggregation. Microbial exocellular polymers can increase the water-retention properties of minerals, especially the retention of readily available water [124] (see Section II.A). Microbes themselves can create porosities: in vertisols, tubular pores of several microns in diameter are voids left by dead fungi [Cabidoche, 1990 personal communication].

In a stable aggregated structure, at least two kinds of pore spaces occur: intra-aggregate pores provide storage for water and interaggregate pores ensure the circulation of air and water. These porosities constitute different ecological habitats [2], as previously discussed in this chapter (Section III.A). The amount and stability of the protected intra-aggregate microniches are undoubtedly dependent on some stable aggregation (e.g., it was demonstrated that both microenvironments occurred in a vertisol A1 horizon, whereas external microniches were dominant in an unstable and poorly aggregated alfisol A1 horizon [59]).

Management of Microbial Aggregation

What are the possible applications of microbially mediated aggregation for the improvement or preservation of soil structure and fertility? The aggregating effect of microbes can be promoted by classic or more recent agricultural practices, such as the growing of plants that encourage rhizosphere activity, the return of straw and other plant residues to the soil, the addition of animal or industrial organic manures, and the conditioning of the soil with polysaccharides or polysaccharide-producing microorganisms. The conditioning of agricultural soils with polysaccharides directly sprayed on the surface has been considered since the 1950s [342], but this was not practical as the result of the high costs and low persistence of the conditioners. Nevertheless, there is renewed interest in this subject [343–346]. However, application of polysaccharide solutions to soils is likely to be restricted to particular situations, such as irrigated soils [346–348] or slope stabilization [349].

More exciting fields of research involve the inoculation and management in soils of microbial populations to promote their aggregating activity. Polysaccharide-producing unicellular algae (e.g., *Chlamydomonas mexicana* and *C. sajao*) sprayed on soils in the temperate regions grew and improved the stability of the soil aggregates [350–352]. However, only surface stabilization was achieved: increases in aggregate stability and in polysaccharide contents

occurred mainly in the top 2–3 mm of the soil [350,351,353]. There is a potential for soil conditioning with algae, although it appears to be restricted to irrigated farmlands, as algae are very sensitive to water stress. Species of *Chlamydomonas* reproduce well on soil between water potentials of 0.0 and −0.1 MPa [354]. However, the autotrophy of algae is a great advantage, as they do not need supplemental carbon, nor do they compete with indigenous heterotrophic microbes.

Another field of applied research is the management of bacterial populations to promote their production of polysaccharides and their aggregating activity. It is hypothesized that soil carbohydrate levels and aggregation can be controlled by short-term management practices that control N supply and the C/N ratio [Roberson, E. B., S. Sarig, and M. K. Firestone, 1989. Management of polysaccharide mediated aggregation in agricultural soils. Agron. Abst. p. 225]. The C/N ratio and water stress have been shown to control polysaccharide production by soil a pseudomonad [132].

Conclusion

Extracellular polysaccharides are emphasized as constituents of major importance in microbial functions in soils, as they form the interface between microbes and the soil constituents. Extracellular polysaccharides are involved in aggregation of soil particles and in the resistance of microbes to wetting and drying cycles and to high osmotic stress. They may also be involved, through their chelating properties, in the resistance to the toxicity of Al and heavy metal and in metal accumulation at the cell surface (e.g., Ca, Fe, Mn). Many of these properties are concerned with the various levels of structure of polysaccharides, from the primary structure (functional groups) to the tertiary and quaternary levels.

V. GENERAL CONCLUSION

Soil is a place of interactions between microorganisms and soil constituents and where microbial events are localized in microsystems. Physical soil factors are at least as important as chemical soil factors for microbial life. The presence of pores, their size and filling by water and air, determines mobility, competition between microorganisms, and type of dominant microbial function (e.g., aerobic versus anaerobic). Here, microhabitats derived from associations with clay particles are of great importance for soil microorganisms, especially bacteria, in soil.

Knowledge of soil organization and related physical properties allows some general predictions of microbial function. Among the

different levels of soil organization, the aggregate level represents the main microsystem. Even if reality is more complex than a simple distinction between inner and outer positions relative to stable aggregates, it is a convenient way to understand the distribution of microorganisms and their activity.

Different methods exist to determine and study soil aggregation: direct observation, fractionation, or an indirect approach through volumetric moisture curves. These methods can also be used for microorganisms, and good tracers exist, such as ATP or C and N isotopes. Ideally, such methods should be used from the macroscale (macroaggregate) to the microscale, which represents the interface between clay, organic matter, or biopolymers (polysaccharides), and microbes.

Concerning chemical constraints, acidity, which is mainly related to aluminum toxicity, was discussed as an example. Acidity is the main chemical stress in many soils of the world, and its importance is increasing in the remaining parts because of acid rains. However, in chemical stress, heavy metals as well as pesticides also constitute major constraints. It is important to study further the effect of all these factors on microbial life in soil and the remedies that can be applied (e.g., liming, organic matter management, genetic adaptation of microbes and plants) especially for Al tolerance.

Considering soils as places of interactions permits making some general statements. If environmental factors of soil are important for microbial life, microbial life is, in return, a major factor in the soil environment. Microbes are able to transform the physical properties of their microenvironment through aggregation and the chemical properties through weathering (i.e., microbes modify both the structure and the geochemical cycles in soil). Although nutrients were not discussed in this chapter, it is important to consider that microbes can fix elements (N) or weather minerals to extract insoluble elements (P, K, Fe) if agriculture is to become more biological.

All these aspects need to be considered if new orientations have to be devised for the future. The first way could be to enhance the functions of microbes through in situ management of microbial functions. Introduction of microbes into soils is increasing, primarily for rhizobia and mycorrhizae and for biological control of plant diseases. However, inoculation may also be a way to enhance plant growth through plant growth-promoting bacteria (PGR) and to improve bioremediation of excess of nitrite, pesticides, and heavy metals in soil. These aspects represent new trends of research for the future and point out the necessity of better understanding the microbial ecology of soils.

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